An Evaluation of Chemotherapy And Vector Control by Insecticides For Combating Dutch Elm Disease

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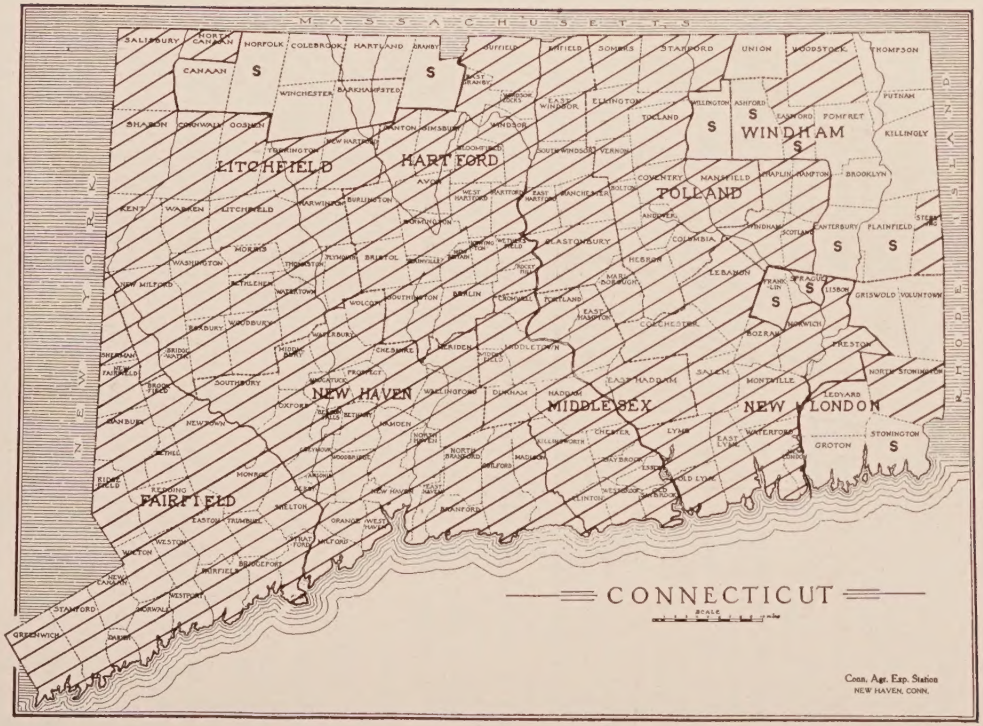

Figure 1. The distribution of Dutch elm disease and *Scolytus multistriatus* in Connecticut. Diseased trees and *Scolytus* have been found in diagonally shaded towns. *Scolytus multistriatus*, principal insect vector of Dutch elm disease in Connecticut, has been found in towns marked with an S.

An Evaluation of Chemotherapy and Vector Control by Insecticides for Combating Dutch Elm Disease

A. E. Dimond, G. H. Plumb, E. M. Stoddard and J. G. Horsfall.[1]

I. Introduction

From its introduction into the United States in 1933, Dutch elm disease has spread steadily and now is found as far west as Denver, Colorado, as far north as Vermont and upper New York State[2] and as far south as Virginia. The bare skeletons of victims of this disease are now an all too familiar sight in southern New England. (See Figure 1 for distribution of Dutch elm disease in Connecticut in 1948.)

With the appearance of Dutch elm disease in the United States, efforts at complete eradication were begun. In 1933 this effort seemed reasonable and there was general agreement that such work should be undertaken. Under the rigid quarantine that was adopted, it was illegal to harbor diseased trees or diseased material from which infection could be spread. Thus, until Dutch elm disease had become established within an area, it was impossible to do research on alternative methods of control should eradicative efforts fail.

By 1940 it had become apparent that Dutch elm disease had become firmly established in the United States. The disease had by that time moved well into Connecticut and diseased trees were being removed less rapidly than new ones became infected. The causes behind the failure of eradicative efforts are now apparent. These were primarily the practical difficulty of diagnosis at a sufficiently early date to make removal effective, the impossibility in a large area of completely removing and destroying bark in which beetles could breed, and of doing this in the short period of time between successive generations of the elm bark beetle.

In 1940, research on alternative methods of controlling Dutch elm disease was begun by the Connecticut Agricultural Experiment Station, and this program has been pursued vigorously since that time. A detailed account of these efforts between 1940 and 1944 has already been published(86). The present bulletin summarizes research on Dutch elm disease between 1945 and 1948.

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[1]Associate Pathologist, Assistant Entomologist, Associate Pathologist, and Director of Station and Chief of Dept. of Plant Pathology and Botany, respectively.

[2]A second area of infection already occurs in Quebec along the St. Lawrence River. This zone now extends south almost to the U. S. border and westward to Ottawa.

II. Chemotherapeutic Studies on Dutch Elm Disease

A. GENERAL CONSIDERATIONS

The revival of interest in chemotherapy of plant diseases began with work by Howard (44, 45) and Howard and Caroselli (46) who, in studying the bleeding canker disease of hard maples caused by *Phytophthora cactorum*, noted that symptoms are in large measure associated with production of a toxin by the fungus. The toxin, translocated from trunk cankers to foliage, there produces the characteristic disease symptoms: wilting and dying of leaves, followed by dying of branches. They isolated the toxin from cultures and demonstrated that diaminoazobenzene dihydrochloride antidotes it. Tomato or maple cuttings, immersed in mixtures of toxin and chemotherapeutant, failed to wilt, whereas test cuttings in toxin alone wilted rapidly. These workers then injected diaminoazobenzene dihydrochloride into maples affected by bleeding canker disease and obtained recovery.

A similar approach has been made to Dutch elm disease by the group at the Connecticut Agricultural Experiment Station. Dutch elm disease is caused by the fungus *Ceratostomella ulmi* (Schwartz) Buis. It grows in xylem tissue, that portion of the tree whose primary function is the conduction of water to the leaves. The disease is a typical vascular wilt. Although the fungus is located in xylem tissue, the principal visible symptoms consist of wilting and browning of leaves, followed by dying of buds and branches. Life history studies of the fungus have shown that it is an organism living almost exclusively inside of its host, save for the short period when it is carried on or in the body of its vectors, the elm bark beetles, *Scolytus multistriatus* and *Hylurgopinus rufipes*. These characteristics dictate the chemotherapeutic approach as a preventive or curative measure in combating the fungus directly.

B. THEORY UNDERLYING THE SELECTION OF CHEMOTHERAPEUTANTS

1. The Toxin Theory

With these considerations in mind, Zentmyer (84, 85) succeeded in showing that nutrient solutions on which *C. ulmi* had grown[1] were capable of wilting tomato and elm cuttings, and Horsfall and Zentmyer (42, 43) extended this discovery to the idea that the toxin might well be antidoted to effect chemotherapy of Dutch elm disease. Further investigation revealed that such toxin, injected into small elm trees, caused many of the typical disease symptoms, i.e., young leaves wilted, died and fell, older leaves curled upward or developed necrotic spots, and stems showed vascular discoloration (86).

[1]Such solutions, from which the fungus had been removed by filtration or centrifuging, will hereafter be referred to as metabolism solutions.

Toxin was produced in quiet liquid cultures of *C. ulmi*, the toxin titer increasing from the sixth day onward. The titer appeared to be related to the composition of the nutrient medium in these studies, despite comparable growth of the fungus. Asparagine was superior to peptone as a nitrogen source for toxin production. Ammonium nitrate and glycine media supported growth of the fungus, but yielded no toxin (86).

a. The toxins of *C. ulmi* in culture

Zentmyer *et al.* (86) and Feldman *et al.* (24) have demonstrated that cultures of *C. ulmi* produce toxin *in vitro*. Toxin inactivation by chemotherapeutants may serve as a means of controlling Dutch elm disease (24, 42, 43). If the chemical nature of the toxin were known, active chemotherapeutants could be predicted, rather than empirically selected. Consequently, studies were undertaken to learn more about these toxic fractions.

(1) COMPOSITION OF THE STANDARD NUTRIENT

It was soon found that *C. ulmi*[1] in shake culture both grew and produced toxin very much more rapidly than in quiet culture. On Zentmyer's (86) nutrient 1, *C. ulmi* in shake culture produced toxin in a four-day period equivalent to that produced in four to six weeks in quiet culture. A careful comparison was then made of the kind and degree of symptom expression by tomato and elm cuttings immersed in culture filtrates from quiet and shake cultures. Even after filtrates had been fractioned, no differences were found. Because of this, shake cultures were used in the studies on fractionating culture filtrates that follow.

In quiet culture, the fungus predominantly forms mycelium; in shake culture, growth is by budding. The change from quiet to shake culture produced a change from an alcoholic to a non-alcoholic metabolism. Others have noted that changes in the environment will bring about the change from mycelial to budding habit in *C. ulmi*. Thus, Tyler and Parker (71), when they varied temperature at which the cultures were maintained, noted that below 18° C. the habit is principally yeast-like, but that above this temperature, more and more mycelium is produced. Boudru (7) concluded that the pH at which the nutrient medium is maintained determined whether the fungus assumed a budding or a *Cephalosporium* habit. Evidently the metabolic pattern of the fungus can be modified by nutrients, oxygen tension, temperature and other environmental changes.

The well-known behavior of yeast at high and low oxygen levels is similar. At low oxygen levels, the fermentation is alcoholic; at high levels, respiration is aerobic. If toxins of *C. ulmi* are associated with the alcoholic fermentation or its aerobic alternate, yeast should also produce similar toxins. Consequently, metabolism solutions from

[1]The culture of *C. ulmi* used in these studies was an isolate from a diseased elm tree, made in 1946. It was periodically inoculated into elm trees and recovered again during the course of these studies.

Saccharomyces cerevisiae, obtained from quiet and shake cultures, were tested on tomato and elm cuttings. Neither filtrate contained toxins.

Moreover, the volatile distillate from quiet cultures of *C. ulmi* which contains the alcohol present, was non-toxic to tomato cuttings. Thus, the suggestion by von Linden *et al.* (51) that alcohol is a toxin in Dutch elm disease can be ruled out.

Several nutrient media were prepared, containing as nitrogen sources asparagine, peptone and a hydrolysate from casein (Casamino acids). *C. ulmi* was inoculated into these nutrients in shake culture. After the fungus had been removed by filtration and centrifuging, tomato and elm cuttings were placed in aliquots of these filtrates. As judged by severity of symptoms on them, the metabolism solution containing the casein hydrolysate was richest in toxin, followed by asparagine and peptone.

Pyridoxine has been shown to be an essential metabolite for *C. ulmi* (11, 55, 56). When this material was substituted for yeast extract in nutrient media (with due increase in casein hydrolysate to compensate for the amino nitrogen in yeast extract), the fungus grew poorly. Thus, yeast extract was used in the standard nutrient instead of pyridoxine.

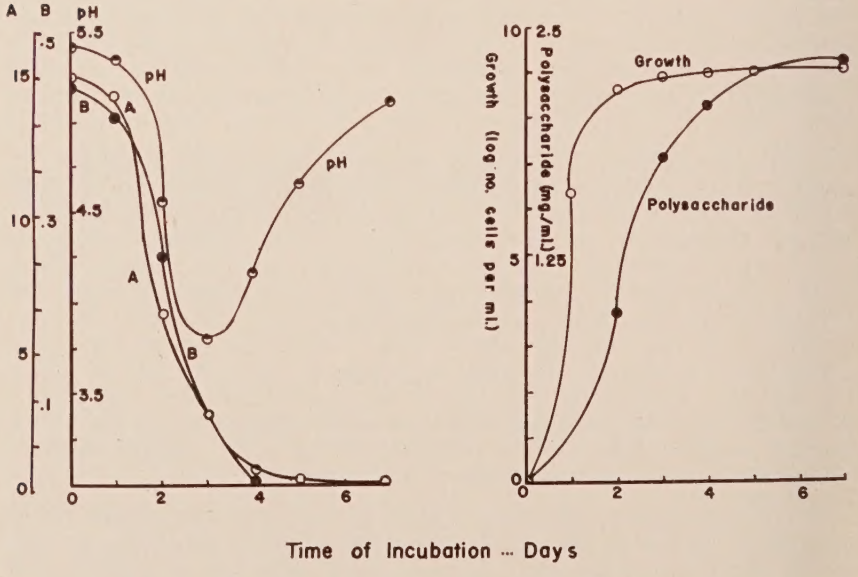


Figure 2. Growth and metabolism of *Ceratostomella ulmi* on standard nutrient solution. Growth was by budding and is expressed as the logarithm of the number of bud cells per ml. Amino nitrogen (Curve B) and reducing sugar concentrations (Curve A) are expressed as mg. per ml. Polysaccharide was determined as reducing sugar after hydrolysis and is expressed as mg. per ml. of reducing sugar.

In order to place metabolism studies on a more secure basis, the amounts of glucose and casein hydrolysate were varied until the relative rates of utilization of reducing sugar and amino nitrogen were the same. This was determined by making analyses on parallel cultures from time to time as fermentation proceeded (19) (Figure 2). The composition of the nutrient medium adopted on the basis of these studies was: Casamino acids, 1.5 g.; yeast extract, 1 g.; KH_2PO_4, 1.5 g.; $MgSO_4$, 1 g.; glucose, 15 g.; trace elements from Hoagland's (36) A to Z mixture, 10 ml., and water to make 1 liter.

Concurrently with these analyses, counts of cell number in cultures were made in a haemocytometer, and pH of nutrient solutions was determined (Figure 2). It can be seen that in this nutrient, growth of cells had almost ceased by the time the nutrient was exhausted of reducing sugar and that no other source of carbohydrate was available further to increase cell number. The figure also shows that the minimum pH value was attained timewise when the glucose became exhausted. Beyond this point, organic acids presumably were utilized and the pH rose again (19). The course of pH in cultures having glucose in excess of balance with respect to nitrogen is downward with time, finally levelling off in the neighborhood of pH 3 and remaining there until and if glucose becomes exhausted.

(2) STUDIES ON METABOLISM SOLUTIONS

(a) Bioassay Methods

At each step in separation of toxic fractions from culture filtrates, the toxicity was measured in terms of the response on tomato cuttings, and occasionally on elm cuttings. The top of a four to six week old tomato plant was severed from its root and quickly plunged under water. Immediately a second cut was made under water. After sufficient time had been allowed for pressure differences inside and outside of the stem to come to equilibrium, the cuttings were immersed in the test solution. Elm cuttings were prepared similarly from one year old seedling trees.

Because the several fractions elicited a variety of responses in tomatoes and elm, no effort was made at this stage to make the technique quantitative. Its principal service was to tell whether or not fractions acting differently upon the plant had been separated.

(b) Separation and Purification of Toxic Fractions

The outline of the procedure used in separating toxins is shown in Figure 3. At all stages in these separations, great care has been taken to assure that the methods were mild so as to prevent altering compounds present in the original metabolism solution.

(i) Properties of unfermented medium and its components

Tomato cuttings were immersed for 36 hours in the standard nutrient and in solutions of the separate components. Table 1 presents the response of the cuttings.

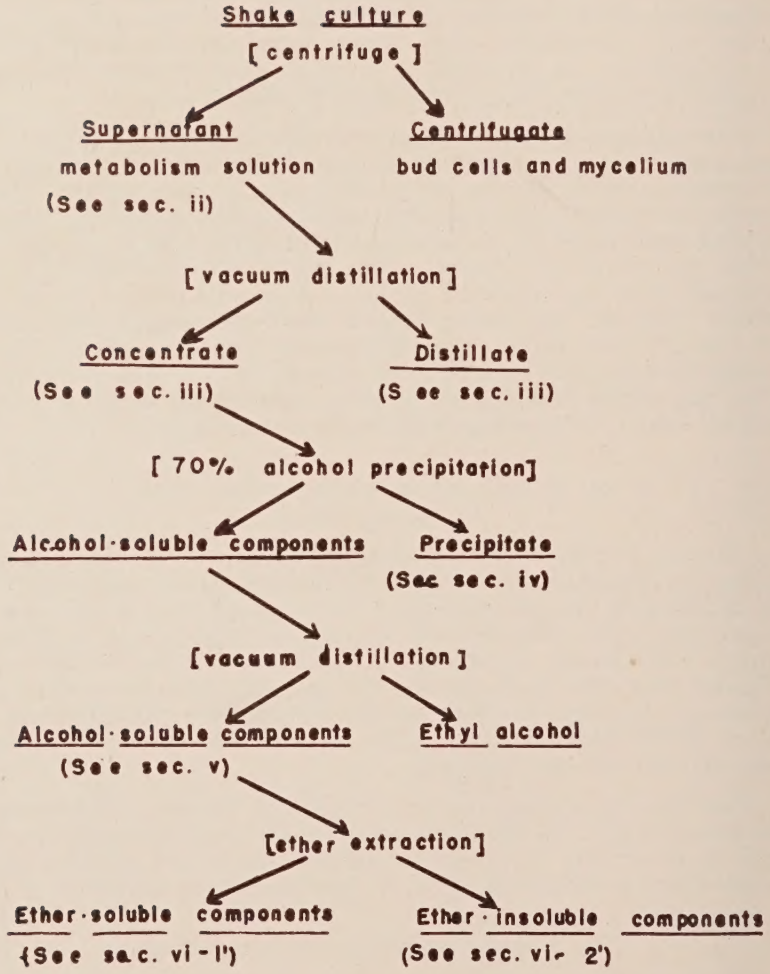


Figure 3. Outline of procedure used for separating phytotoxic fractions from culture filtrates of *C. ulmi* and index to portion of test in which phytotoxic behavior of fraction is discussed.

TABLE 1
Response of Tomato Cuttings to Standard Nutrient and Its Components

Chemical	*Conc. g/l*	*Symptoms*
Full nutrient		none
Glucose	25	marginal upcurl oldest leaflets
	12.5	none
Casamino acids	1.0	slight wilt; otherwise normal
Mineral salts	(see p. 9)	none
Yeast extract	0.5	general yellowing oldest leaves, no wilting or spotting

Neither the separate components nor the entire medium produced symptoms in tomatoes resembling those produced by the toxic fractions of culture filtrates on which *C. ulmi* had been grown.

(ii) Properties of filtered metabolism solutions

Metabolism solutions freed of the fungus by Seitz filtration from four-day cultures of *C. ulmi* usually produced wilting of tomato cuttings rather rapidly, the time for wilting depending upon humidity and the amount of sunlight during the period of test. Leaflets of cuttings frequently showed a pronounced upward curling at the margins followed by marginal drying and dying. Necrotic spotting of the interveinal tissue of leaflets has also been observed. More occasionally, the stem immersed in the culture filtrate collapsed completely and, in such circumstances, there is usually no pronounced leaf symptom. In such a case the filtrate is probably not translocated. Collapse might arise from osmotic effects alone. As we shall see (p. 14, the ether soluble fraction), these tests have conclusively ruled out osmotic effects as the cause. Upcurling of leaves and necrotic spotting cannot be osmotic in origin.

The filtered metabolism solution retains its ability to affect cuttings adversely despite boiling at 100° C. and autoclaving at 15 pounds pressure for 20 minutes. Zentmyer *et al.* (86) have already noted that the toxins are heat stable.

As a routine procedure, bud cells of *C. ulmi* were removed by centrifuging rather than by Seitz filtration, since cells of the fungus were practically all removed in this way and rendered inactive by other procedures. The supernatant behaved identically with filtrates which had been Seitz filtered.

(iii) Properties of the concentrated culture filtrate

Samples of the metabolism solution were distilled and the distillate tested for activity. None was found whether the distillation was at atmospheric pressure or *in vacuo* with maximum distillation temperatures of 38° C. The toxins are, therefore, non-volatile. The distillate was neutral in reaction.

After removing most of the cells by centrifuging, the metabolism solution was reduced in volume by vacuum distillation. In this way one liter of solution was reduced to about 50 ml. and again centrifuged to remove all dead cells and mycelium. At this point a recheck of toxicity to tomato cuttings showed a very marked concentration of toxic principle. By diluting an aliquot back to its original volume and testing again for toxicity to tomato cuttings, it was found that very little of the toxic agent had been lost in these operations. The distillate, being non-toxic to cuttings, was discarded.

(iv) Properties of the alcoholic precipitate

Hodgson *et al.* have isolated a polysaccharide from cultures of the crown gall bacterium (40) and demonstrated that this is capable of inducing a characteristic wilting in tomato cuttings (37, 41). The symptoms displayed by tomato cuttings immersed in culture filtrates of *C. ulmi* resembled closely those described by Hodgson *et al.* (37, 38, 39, 41). Therefore, a polysaccharide was suspected of being present in the culture filtrate. To separate it, the concentrated metabolism solution was brought to a 70 per cent alcoholic concentration (40). A voluminous white precipitate was obtained. Higher concentrations of alcohol yielded very little additional precipitate.

Two entities were sought in this precipitate. On one hand, there was presumptive evidence for a polysaccharide. On the other, the pH of the metabolism solution rose after alcohol precipitation and, presumably, acid was being removed in the process. Of the organic acids formed by fungi from sugars, the one most likely to be precipitated by alcohol was gluconic acid.

(1′) *Separation of polysaccharide*

The alcoholic precipitate was separated by centrifuging. It was purified by successive solution in water and reprecipitation from alcohol (40). Successive batches of this product varied slightly in their response to IKI solutions; some of them produced no color reaction, whereas others developed a slight pinkish color. All such precipitates contained negligible reducing substance initially but, after acid hydrolysis, reducing sugars were present in quantity. These are the characteristics of a polysaccharide.

This polysaccharide was partly broken into reducing sugars when mixed with Takadiastase or other amylase preparations and allowed to stand for a short time at room temperature. It is not a simple polysaccharide, since Pectinol, a commercial pectic enzyme, also brings about partial reduction.

Tomato and elm cuttings immersed in this fraction showed a very strong upcurling of leaf margins, followed by flaccidity, drying and dying of marginal tissues. When such preparations were diluted to the concentration in the original culture solution, tomato cuttings showed strongly upcurled leaflets followed by marginal withering. Very similar leaf symptoms frequently appear on diseased elms in the field.

These symptoms are indistinguishable from those produced by the polysaccharide from the crown gall bacterium, as described by Hodgson *et al.* (38, 41). They are also produced by soluble starch solutions. Apparently, then, a polysaccharide is produced in culture solutions of *C. ulmi* (19).

An estimate was made of how rapidly polysaccharide is formed in shake cultures (Figure 3). This was done by measuring the amount of reducing substances in the nutrient medium before and after acid hydrolysis. The difference between these values was taken as an estimate of polysaccharide content.

(2′) *Separation of gluconic acid*

The precipitate from 70 per cent alcohol was assumed to contain gluconic acid. To isolate it, the crude precipitate was removed and redissolved in a low volume of water. Sufficient $Ca(OH)_2$ was added to render the solution alkaline, which precipitated a calcium salt. This salt was filtered off, suspended in a small amount of water, and treated with ammonium oxalate and ammonium hydroxide. The resulting precipitate of calcium oxalate and Ca-polysaccharide complex was removed by filtration and discarded. The filtrate, assumed to contain calcium gluconate, was acidified and then reacted with phenylhydrazine hydrochloride (17). This yielded a crystalline product, which on recrystallization had an uncorrected melting point of 200°C. The melting point of the phenylhydrazide of gluconic acid is reported as 200 to 202° C. (17).

Gluconic acid was prepared from a sample of calcium gluconate of known purity. The calcium salt and the free acid were tested on tomato cuttings in 1 per cent aqueous solution. These produced a severe interveinal burning of tomato cuttings, but were not phytotoxic at concentrations of 0.1 per cent. The symptoms characteristic of gluconic acid were never seen on tomato cuttings placed in unconcentrated metabolism solutions. The concentration of gluconic acid in such solutions was not determined. However, gluconic acid cannot be eliminated as a toxin responsible for symptoms of Dutch elm disease (see p. 15).

(v) Properties of the alcohol-soluble fraction

This fraction was supernatant after centrifuging off the alcoholic precipitate. Alcohol was distilled off *in vacuo* leaving a straw-brown colored solution which contained reducing sugars. There was a slight ester-like odor after the alcohol had been removed. This was not present in the concentrate to which alcohol was added. On tomato cuttings the concentrate, which had been freed of alcohol, produced necrotic spotting in the interveinal areas of leaflets and occasionally it caused collapse of the stem or severe wilting.

(vi) Properties of the ether fractions

This concentrated, alcohol-soluble fraction was extracted continuously for 20 hours with ethyl ether. The nature of the compounds

extracted with ether and those which are ether-insoluble is unknown. Probably the ether-insoluble fraction represents a mixture while the ether-soluble fraction is relatively pure.

(1′) *The ether-soluble fraction*

The ether-soluble fraction was freed of ether by evaporation. Usually, the fraction was water-soluble and non-phytotoxic. In one experiment, however, as the ether evaporated, a bulky white flocculant precipitate separated and more was thrown down as the solution cooled. A test with an ether solution of this precipitate with $FeCl_3$ indicated that it is probably not a phenol. After diluting tenfold with water, the saturated aqueous solution had a pH of 2.65. This represents a concentration seven times greater than that in the original culture filtrate. Tomato cuttings placed in this responded by complete collapse of the stem tissue, the leaves remaining normal. On diluting 1 to 100 with water (0.7 of original concentration in metabolism solution), collapse of the stem was restricted to the terminal centimeter, the cutting otherwise remaining normal. Because its concentration was less than in the original culture filtrate, osmotic effects can be eliminated as the cause of collapse.

(2′) *The ether-insoluble fraction*

The ether-insoluble fraction was diluted tenfold with water, its pH being 3.68. Tomato cuttings immersed in this (seven times the concentration of original culture filtrate) wilted, became yellow, developed necrotic spots in the interveinal areas, and showed upcurling of unwilted leaflets. A duplicate sample of this fraction was precipitated with $Ba(OH)^2$ and brought by this means to pH 6.7. After the precipitate was removed, tomato cuttings showed the same necrotic spotting and upcurling of leaflets.

The ether-insoluble, 70 per cent alcohol-soluble fraction contained some of the original inorganic salts, unutilized glucose, and unused nitrogen materials, together with metabolic products. This fraction became contaminated on standing.

(3′) *The ether fractions from filtrates not precipitated with alcohol*

When ether extraction was carried out *prior to* alcohol precipitation, the ether-insoluble fraction consisted of a turbid top layer, containing polysaccharide, and a clear lower layer. This fraction caused necrotic spotting and the marginal withering characteristic of the polysaccharide in tomato leaflets.

The ether-soluble fraction, after evaporation of ether, had no ester odor, reacted with phenylhydrazine to produce a dense, yellow-brown precipitate which was lost on attempted recrystallization. The reaction product may have been glucosazone or the phenylhydrazide of gluconic acid. A duplicate sample, diluted 1 to 10, caused no wilting of tomato cuttings, but collapsed the stem completely out to the midribs of the leaves, and caused severe veinal bleaching and

necrosis, possibly due to gluconic acid (see p. 13). This concentration represented a 7-fold increase over that in the original metabolism solution. In dilutions of 1:100 (0.7 of original concentration) tomato stems were collapsed only in the terminal centimeter, but otherwise were normal.

b. Discussion of cultural studies

(1) POLYSACCHARIDES AS TOXINS

Hodgson *et al.* (37-41) isolated from cultures of the crown gall organism a polysaccharide which causes wilting of tomato leaflets. By analytical methods they have determined that this compound is translocated in cuttings to the margins of leaflets, and by bioassay techniques have shown that the response of cuttings is graded with the amount of polysaccharide absorbed. Extending these studies to other polysaccharides and polymers, they have shown that whether the material is translocated or not is related to molecular weight, the amount moving to the leaf margins decreasing as molecular weight increases. The manner of symptom expression in such cuttings varies with molecular weight of the polymer as a result of these relations. This work has indicated that such polymers all seriously interfere with water economy of the exposed cutting, and the interference is likewise graded in proportion to the dosage of toxin absorbed.

In the present work, it has been shown that *C. ulmi* in culture produces a complex carbohydrate which affects tomato and elm cuttings similarly. In fact, on these cuttings, one cannot distinguish between the symptoms produced by soluble starch solutions and the complex carbohydrate from culture filtrates of *C. ulmi*. Carefully controlled studies have shown that the amount of this carbohydrate in undiluted culture filtrates is adequate to cause typical response by either tomato or elm cuttings. Purified preparations can likewise be diluted back to the concentration in the original culture filtrate to produce these effects. It is important to bear in mind that the culture of *C. ulmi* used in these studies was an isolate from a diseased tree, producing hyaline mycelium and fruiting bodies, and capable of producing typical symptoms of Dutch elm disease in inoculated elms.

Recently, Feldman, Caroselli and Howard (24) have carefully studied the possible role of this polysaccharide as a toxin in Dutch elm disease, and have concluded that it is unimportant in pathogenesis. No correlation was found between polysaccharide production in liquid culture and wilting. Polysaccharide formation could be prevented by proper buffering of the nutrient and, when this was done, highest toxin titer was obtained. Under the conditions of their studies, apparently another, more potent toxin is produced, but only at low oxygen tensions (24). This is rendered inactive when culture filtrates are adjusted above pH 7 with NaOH or $Ca(OH)_2$.

This excellent work raises two questions concerning the present investigations. These are concerned with (1) the amount of polysaccharide produced and (2) the relative amounts of the several phytotoxic

components in culture filtrates produced in shake culture (high oxygen tension) and in quiet culture (low oxygen tension). Under the conditions of our own studies, sufficient polysaccharide has been produced to induce wilting in cuttings, both in shake and in quiet culture. The amount of wilting has been consistently proportional to the amount of polysaccharide to which cuttings were subjected. Careful comparisons have been made of shake and of quiet culture filtrates before fractionation. Tomato cuttings have not distinguished between them in our tests.

How may this difference be resolved? Feldman *et al.* (24) have emphasized that a number of environmental conditions may affect the amount of toxins formed, and Zentmyer *et al.* (86) and the present work have also pointed out that composition of the nutrient may affect toxin titer. Such factors, affecting toxin titer or types of toxins, may not have been identically controlled in these two studies. On this basis, one could account for the differences.

Genetic differences between strains of *C. ulmi* may likewise account for differences in these two cases. Tyler and Parker (71, 72) have indicated that strains of *C. ulmi* exist which differ widely in their pathogenicity. Presumably, they would also differ in their ability to produce toxin, as Wellman(76) and Haymaker (35) have reported for strains of *Fusarium lycopersici*, the fungus causing Fusarium wilt of tomato.

(2) THE NUMBER OF TOXINS INVOLVED

Since the time of Hutchinson (47) at least, the thought that fungus parasites produce toxins has been gaining in importance. As these studies relate to vascular wilt diseases, toxins have been assigned a major role in pathogenesis. The inference drawn from many of these studies is that one toxin is primarily culpable in each case.

Only occasionally has the suggestion appeared that more than one toxic fraction is isolable from culture filtrates of pathogenic fungi. White (77) in 1927 noted this relation with *Fusarium lycopersici* for tomato wilt. More recently, it has been noted in relation to *C. ulmi* (19) and for *Fusarium oxysporum* var. *nicotianae* (81). The data presented here have indicated this clearly, with the difference that apparently *C. ulmi* produces not two, but possibly several, toxic fractions, and that among these are some that act at the dilution of the original culture filtrate.

If it is assumed for the moment that the toxic fractions produced in culture are all produced in the sick plant and can be assigned a role in pathogenesis, the toxin theory will account for the multiplicity of symptoms expressed by the diseased plant. In relation to chemotherapy, however, this aspect of the toxin theory introduces serious complications. The several toxic fractions have quite different properties, which permit their separation from culture filtrates, and produce different symptoms on test cuttings. They can be presumed to differ chemically. They will, accordingly, need to be neutralized, each by its own antidoting agent.

(3) TOXINS FROM CULTURES *in vitro* AND IN THE DISEASED HOST

In most of the cases reported in the literature, fractions toxic to the host have been obtained only from cultures *in vitro*. In a few cases, efforts have been made to relate the properties of such a toxin to pathogenicity of strains or to virulence in hosts. Thus, Wellman (76) showed that culture filtrates from *Fusarium lycopersici* affect Bonny Best and Marglobe tomatoes in the order of their susceptibility to Fusarium wilt. He also demonstrated that toxin production by virulent and mild strains, at least in the first 30 days of growth on liquid media, was in the order of their virulence. Gottlieb (33, 34), on the other hand, considered that if a toxin exists, it should be isolable from the diseased host. He was successful in isolating a toxin from Fusarium-infected tomatoes which induced wilting in healthy tomatoes. Only Wolf (80) has examined toxins produced by one fungus in culture media and in the diseased host. In this case culture filtrates from *Phytophthora nicotianae*, cause of black shank of tobacco, produced the same wilting symptoms in tobacco as extracts from black shank lesions. This is the closest approach yet made to proof of identity of toxins produced *in vitro* and *in vivo*, and no chemical evidence is available even in this case. The evidence along these lines as concerns *C. ulmi* is exceedingly meager and the present study has done little to clarify this aspect of the problem.

Whether toxins *in vitro* and *in vivo* are identical or not becomes very important in light of the possibility that one is no longer dealing with a toxin but with several toxins. If strain of *C. ulmi*, composition of nutrient, or environment can affect type and amount of toxin formation, it becomes very important to know whether these toxins have an important role in pathogenesis before using them as a basis for screening chemotherapeutants.

(4) TOXINS AND RESISTANT HOSTS OF *C. ulmi*.

Ulmus pumila and the Buisman elm are immune to Dutch elm disease, or at least are highly resistant to it. *C. ulmi* apparently can invade these resistant hosts and grow freely in them. Thus, when Wollenweber (83) and Sibilia (59-61) inoculated *U. pumila* with *C. ulmi*, it showed no external symptoms of disease. The wood, however, showed vascular discoloration, and yielded *C. ulmi* in recovery cultures. Buisman (10) and Smucker (63) have shown this relation to exist, not only for *U. pumila*, but also for the Buisman elm. Smucker (64) has also shown that this situation occurs in apple trees which have been inoculated with *C. ulmi*.

All of the toxic fractions which have been isolated from *C. ulmi* produce toxicity symptoms on a wide variety of hosts. Thus, they produce symptoms on cuttings of tomato, a test plant which has been used in all toxin studies on *C. ulmi*, and act with equal vigor on snapdragons (86), twigs of maple (86), and on Siberian and American elm. Action on such a wide range of plant tissues would indicate general toxicity.

What may we conclude from these facts? The fungus causing Dutch elm disease grows in resistant species of elm and produces vas-

cular discoloration in them. Toxic culture filtrates of this same fungus produce symptoms on resistant species. One must conclude at least that toxins associated with foliage symptoms are not formed in trees belonging to resistant species. If these are not formed in resistant species, then the conditions for toxin formation *in vivo* must be rather more specific than we have appreciated in the past. If this is so, conclusions based on toxin production *in vitro* may be seriously called into question, until placed on a firm foundation by studies in parallel, carried on in inoculated trees of resistant and susceptible character.

2. Water Economy and Dutch Elm Disease

a. Behavior of diseased elm wood

Many of the symptoms of Dutch elm disease are those of a plant which is short of water. Leaves wilt, shrivel, turn brown, and fall from the tree. The leaves on a healthy elm branch will display these same symptoms if the branch is cut from the tree and, after a few minutes, the cut end placed in a pail of water (see p. 21). In this case air, introduced into the vascular tissue, has prevented more water from rising in the stem. There is some evidence that a large part of the syndrome of Dutch elm disease can be accounted for in terms of a water shortage in the leaves.

Broekhuizen (8) has indicated how such water shortage may arise. As a result of histological studies, he showed that water-conducting cells become occluded by tyloses and gums as involvement by Dutch elm disease increases. This is a long-term effect, inasmuch as gum plugs and tyloses require several days to form. The vascular browning characteristic of diseased wood is frequently attributed to such plugging. Such effects may be caused by the elm in response to the fungus itself or to a toxin. The important point, however, is that these effects are irreversible. Once a vessel is occluded, it cannot be reopened by presently known techniques. This would seem to indicate on theoretical grounds that a chemotherapeutant must be introduced prior to such changes if it is to prevent water shortage effectively and become adequately distributed in order to prevent further involvement.

Such plugging should result in decreased water movement in diseased stems. Zentmyer *et al.* (86) have compared the amount of water moving through diseased and healthy sticks under constant pressure head. Significantly less water flowed through diseased sticks than through healthy ones. Rate of water flow through sticks from diseased trees decreased with time after inoculation of the tree with *C. ulmi*, and significant decrease had occurred within two days. Decrease of flow so soon after inoculation cannot be attributed to mechanical occlusion by tyloses and gum plugs. Zentmyer *et al.* (86) further observed that in sticks from trees injected with culture filtrates of *C. ulmi*, there was a decreased flow of water one week after injection. Interference by toxins in this way must be by other means than gross mechanical obstruction of the water columns. Yet the site of interference is clearly in the wood rather than in the leaves. How can such interference come about? The only ready way of accounting for

it in light of available information is in terms of a polysaccharide or other water-binding material which, in highly hydrated form, lodges in the water-conducting cells and reduces flow through them.

b. The effect of toxic fractions from *C. ulmi* on water economy

The above reasoning leads to an interesting question. What is the effect of the various toxic fractions on the movement of water through plant cuttings? Evidence on this point was accumulated, using tomato cuttings for test plants. In these experiments the several fractions were used at concentrations in the original metabolism solution. A sample of the fraction was placed in a vial. Weights were taken of vial plus solution and cutting, at the start and at various time intervals during the course of the experiment. The effect of the several fractions on the water economy may be seen in Table 2. These tests were carried out in an air conditioned room at 30° C. and at 40 per cent relative humidity. The values are corrected for evaporation from a blank vial containing test solution. Effects have been confirmed in repeated tests.

TABLE 2

Effect of Toxic Fractions from Cultures of *C. ulmi* on the Water Economy of Tomato Cuttings

Fraction	*Wt. cutting in gms. at start*	*Volume absorbed (ml.)*	*Rate water absorption ml./hr./gm. wt. cutting 44 hrs.*
Vacuum distillate	6.0	23.5	0.090
Undistilled liquor	4.2	5.0	0.028
70% alcohol ppt.	2.9	3.0	0.043
Alcohol-soluble	5.2	10.0	0.044
Water control	5.0	17.5	0.080

As indicated by Table 2, there is very serious impairment of the water economy of tomato cuttings by three of the fractions: the undistilled liquor, the alcoholic precipitate which contains the polysaccharide, and the alcohol-soluble fraction which contains an unknown toxin or toxins. There is greater impairment of water absorption in the undistilled liquor because it contains both polysaccharide and the alcohol-soluble fraction.

Because of the design of this test, we cannot determine whether the site of water interference is in the water-conducting columns themselves or in the leaves. Yet the fact is clear that water movement has been seriously reduced and that it is brought on by certain of the toxic fractions from *C. ulmi*. It appears long before tyloses or gum deposits could be formed in response to injury to the fungus, or to toxins. In other words, the toxic fractions interfere with water movement in their own right and do so within a few hours of being introduced. The host response to these materials by tylose or gum plug formation requires much longer than this (38). Thus, vascular discoloration requires several days to appear.

A toxin consisting of polysaccharide could produce interference with water economy either in stem or leaf tissue. As already discussed, Hodgson *et al.* (37-41) have indicated how polysaccharides of low molecular weight may interfere by being translocated to the leaf tissue and there competing for water with spongy parenchyma cells. Those of higher molecular weight may fail to be translocated, because of their size and solubility, and produce blocking of the water-conducting columns. Compounds of intermediate size could act in both ways. Such blocks would not be evident in histological examinations of the sort used by Broekhuizen (8), unless special staining methods were used.

c. Properties of *C. ulmi* in relation to water economy of the elm

Water movement through elm wood may be quickly and seriously reduced by spores or bud cells of *C. ulmi*. Buisman (10) and Banfield (1) have both noted that the spores and bud cells of *C. ulmi* are smaller in diameter than elm wood vessels. Banfield has demonstrated experimentally that spores of fungi causing vascular wilts in elm may be introduced at the base of a healthy tree and recovered from the apex in a very few hours. Such bud cells or spores, apart from their importance in spreading a localized infection, could and probably do interfere with water movement by becoming lodged and forming effective blocks, similar to those made by tyloses. Vessels in elm are very long at the base of the tree and decrease in length gradually toward the apex. Thus, basal infections are more important in this respect than apical ones.

Tyler and Parker (71) have shown experimentally that the growth habit of *C. ulmi* at temperatures below 18° C. is predominantly of the budding type. Thus, the postulated mechanism for water interference by the fungus itself is in line with the facts known experimentally.

d. Water relations of Siberian and American elm

Leaves on cut twigs of American elm wilt rapidly and irreversibly. American elm seems almost unique in the facility with which it develops air lock in the vessels. The only way in which this can be overcome is to cut off the twig and immediately recut the basal end under water. Immediate plunging of the cut end under water is not sufficient. This phenomenon led us to speculate upon the relative susceptibility of Siberian and American elm to physiological wilt.

Our own studies and those of Buisman (10) have indicated that the resistance of *Ulmus pumila* cannot be accounted for on anatomical grounds. Vessel length and diameter are comparable between the two species.

Experiments were undertaken to compare these species with respect to their water economy. Two examples will illustrate the differences noted. In one experiment two twigs each of American and Siberian elm were cut under water and transferred to a flask containing tap water. Other pairs of twigs were simply cut off and allowed to

stand for varying times in air before being transferred to water. Response of these twigs after the times noted was recorded in three categories: fresh, wilted and dried. Index values of 0, 5 and 10, respectively, were assigned to these responses. Results are shown in Table 3.

TABLE 3

Relative Susceptibility of Siberian and American Elm to Physiological Wilt

Treatment	*Index numbers[1] of two twigs each after times noted*					
	1 day		*3 days*		*6 days*	
	Sib.	*Amer.*	*Sib.*	*Amer.*	*Sib.*	*Amer.*
Cut under water	0	0	0	0	0	10
Cut and transferred	0	0	2.5	10	5	10
10 minutes in air	0	0	2.5	7.5	10	10
1 hour in air	0	0	10	7.5	2.5	10
2 hours in air	0	0	2.5	10	5	10
12 hours in air	5	10	5	10	7.5	10
24 hours in air	5	10	5	10	10	10
30 hours in air	10	10	10	10	10	10

[1]Maximum index = 10

These responses indicate a marked difference in susceptibility of American and Siberian elm toward physiological wilt.

A second, independent measure of this response was obtained by removing single leaves of these species, immersing them in graded concentrations of sucrose solution, and obtaining the course of wilting with time. Index numbers were assigned to response as in the first experiment and responses on the 10 leaves of each species per solution were summed. These responses are indicated in Table 4.

TABLE 4

Response of Siberian and American Elm Leaves to Graded Concentrations of Sucrose with Time

Concentration of sucrose	*Index numbers[1] of ten leaves after times noted*									
	1 day		*2 days*		*3 days*		*6 days*		*8 days*	
	Sib.	*Amer.*	*Sib.*	*Amer.*	*Sib.*	*Amer.*	*Sib.*	*Amer.*	*Sib.*	*Amer.*
40%	0	100	0	100	85	100	90	100	100	100
20	0	80	0	100	0	100	10	100	30	100
10	0	50	10	100	10	100	25	100	40	100
5	0	15	0	90	0	100	0	100	0	100
2	0	0	0	90	0	100	0	100	0	100
1	0	10	0	90	0	100	0	100	0	100
water	0	15	0	80	0	100	0	100	0	100

[1]Maximum index = 100

From these data a rather clear-cut case may be made for the greater resistance of Siberian elm to physiological wilt. The difference in susceptibility to water-shortage is in the same direction as the difference in susceptibility to *C. ulmi*. Note that this fungus is parasitic on both species; it is pathogenic only on American elm.

e. Summary

We may now summarize the conclusions to which these studies have led us. Siberian elm is resistant and American elm is susceptible to the presence of *C. ulmi* in its woody tissues. But toxins of *C. ulmi* can readily be shown to be non-specific in nature and will wilt diverse test plants such as tomato, snapdragon, maple and elm cuttings, whether of Siberian or American elm. Therefore, if toxins play a dominant role, they should cause symptoms of disease in Siberian elm. If toxins responsible for damaged water economy are not formed in Siberian elm but are in American elm, the conditions for toxin formation are highly specific. In this case, *in vitro* studies of toxins may not be valid unless it is shown that conditions for their formation are the same in the host and in culture.

It seems more likely that since the fungus grows in both hosts, it interferes with water transport in both. The demonstrably higher resistance of Siberian elm to physiological wilt may suffice to carry it through attack by the fungus under conditions which cause American elm to succumb.

There appear to be two effects on water economy brought on by *C. ulmi* in elms. One appears to be toxin-induced, appears rapidly, and causes water movement to drop approximately in half (Table 2). The other is a result of tylose and gum formation in vessels, appears slowly, is very severe indeed, and may cause water movement to drop to 1 per cent of its original value (86). We do not know yet whether the latter is toxin-induced or is a host response to the fungus itself.

It is open to question whether the water shortage brought on by toxins in the early phases of infection may cause death of the tree. We cannot definitely ascribe to toxins the irreversible and severe interference with water movement brought on by formation of tyloses and gum plugs. These facts are of consequence in choosing a method of selecting chemotherapeutants and in indicating the likelihood that curative treatments may be found.

3. Implications Concerning the Selection of Chemotherapeutants

The foregoing experimental work and discussion have resulted in isolation and identification, not of a single toxin, but rather of several toxic fractions, which are produced in culture. In the following evaluation, we shall assume that all, or at least that more than one of them, are involved actively as toxins in Dutch elm disease. This finding has definite implications with respect to chemotherapy.

a. Limitations of inactivating one or several toxins

(1) SINGLE TOXIN

If but one toxin is formed and compounds can be found which inactivate it and which act *solely* by inactivating toxins, there is strong possibility that a disease will be alleviated. Toxin inactivation alone, however, is likely to be inadequate to control disease. Thus: the fungus produces the toxin: a therapeutant neutralizes the toxin. The fungus is still free to produce more when the therapeutant is exhausted.

If toxins are responsible for the predominant part of water interference in Dutch elm disease, then their inactivation as they are formed becomes important in preventing serious water shortage in the plant. But, as already noted, such compounds must be introduced prior to interference if (1) they are to save the plant from water shortage and (2) if they are to be adequately distributed in the plant. Adequate distribution prior to blocking of the water columns is necessary because the fungus can move freely from point to point in the tree and establish new infection pockets at any time.

If, on the other hand, the formation of gums and tyloses by the elm is a response to the fungus and not to toxins, the water shortage brought on by toxins is not nearly so severe as that brought on by occlusion of the water columns in the tree. Toxin inactivation in this case does not rectify or alleviate the trouble which may eventually kill the tree.

(2) MANY TOXINS

If several toxins are formed by the fungus, as seems likely from the above studies, the problem becomes more complex. Presumably each toxin acts in the plant in its own way to produce its part of the syndrome. Whether all toxins must be inactivated or not depends upon how they severally act upon the tree. If more than one is exceedingly important, and they differ chemically, then each must be inactivated by a separate therapeutant. Each chemotherapeutant must be adequately distributed in the tree. Each must adequately inactivate its toxin throughout the tree. The chances for this become very much less when more than one therapeutant must be introduced into the tree than when only one need be used.

b. Modes of chemotherapeutic action and the non-specific approach

Stoddard and Dimond (70) have discussed the ways in which a chemotherapeutant may act against a plant disease. They distinguish five modes of action and there are doubtless others: (1) inactivating the parasite, (2) preventing its reproduction in the plant, (3) inactivating toxins, (4) preventing toxin formation and (5) increasing resistance of the host.

Accelerated tests of chemotherapeutic activity can be designed in terms of a mode of action, or tests can be non-specific in this respect.

The non-specific type of test merely seeks to determine whether or not a compound has chemotherapeutic activity. When a series of successful compounds has been accumulated, the mode of action can be determined. To design a screen about a single mode of action will probably result in eliminating many useful compounds.

As a result of the studies detailed above, non-specific procedures for screening compounds as chemotherapeutants have been perfected. These have been designed on the basis of the above reasoning, which is, in turn, a product of the culture filtrate studies reported above. The technique in current use consists of treating plants with the compound under test, inoculating the plant with a pathogene, and noting the incidence and severity of disease on such plants in comparison with checks. This technique does not select compounds on the basis of their mode of action, but only on the basis of whether or not they have activity, however it arises. Details of such studies will be reported elsewhere.

C. PRINCIPLES OF FIELD CHEMOTHERAPY

1. Introduction

At the inception of the present studies, two primary problems required solution to advance the chemotherapy of Dutch elm disease: (1) how to select, both quickly and reliably, chemotherapeutants which will be superior to those now in hand and (2) how to apply the results of such tests to field practice. The first of these problems is well on its way to solution and will be reported elsewhere. How the second problem has been approached will concern us here.

Performance of promising materials (86) was known largely in terms of their behavior in small trees. How to predict their performance on large trees from information based on small ones was unknown. Many other aspects of application, such as dosage rate, timing, and protective versus curative activity, were likewise unknown. Some of these principles underly the practice of chemotherapy and are independent of the compound used. Their evaluation is as important as the compound itself in determining the value to the plant of chemotherapeutic treatment.

In experiments reported by Zentmyer *et al.* (86), a group of compounds was selected which inhibit *C. ulmi* in culture. These were injected into trees inoculated with *C. ulmi*. After eliminating the highly phytotoxic and ineffective compounds, only a few of real promise remained. The best of these were p-hydroxyphenol, hydroquinone and 8-quinolinol sulfate and the corresponding benzoate.

8-Quinolinol benzoate[1] was chosen for these investigations because it had already shown ability to alleviate symptoms of disease for a longer period and with greater consistency than other promising compounds. The principles with which the present experiments were

[1]8-Quinolinol benzoate is sometimes called 8-hydroxyquinoline benzoate or simply oxyquinoline benzoate. For brevity, this compound will be referred to as OQB.

concerned could be investigated with any material which showed reasonable promise as a chemotherapeutant.

2. Methods

a. Application of 8-quinolinol benzoate

When applied as a solution in these experiments, OQB was used in concentrations of 0.1 per cent or 13 ounces per 100 gallons of water, unless otherwise noted. Since this represents a concentration slightly greater than a saturated solution, higher concentrations were not employed. In certain experiments, specifically indicated below, OQB in isopropyl alcohol was diluted to the desired degree in water, the isopropyl alcohol serving as a convenient cosolvent. Such solutions were prepared in conventional hydraulic spray tanks at time of application and all solutions were always used immediately upon preparation.

Except in the specific cases noted below, the studies here reported were all conducted by applying water solutions of OQB to the feeding root zone of the tree and allowing the tree to take up the material from the soil. This method of introducing the therapeutant seems to offer several advantages over trunk injection. Stamm (66) has shown that the flow of water and water solutions through sticks of wood in the longitudinal direction is at least a hundred times greater than that in the radial or tangential direction. In practice this means that solutions of chemotherapeutant injected directly into the trunk of a tree will be predominantly distributed up and down from the point of entry and will be poorly distributed about the circumference or radially inward toward the center. In contrast a chemotherapeutant entering through the roots of a tree from the soil will be uniformly distributed. Chemotherapeutic response has been shown experimentally to be more uniform when a compound is absorbed by the roots from soil application than when injected into the stem. The erratic performance of chemotherapeutants in early trials (86) may have been a result of poor distribution associated with trunk injections.

Test solutions were applied to roots of trees in one of two ways, unless otherwise specified. In early experiments the solution was applied to the surface of the soil over the feeding root area. This is termed surface sprinkling. In later experiments a nozzle, such as is used in liquid fertilization of trees, was employed. In such cases the solutions were pumped into the ground at pressures ranging from 150 to 350 lbs./in.^{2}. In this manner, the solutions were applied from 18 to 24 inches beneath the surface of the soil in the feeding root area, the injection holes being spaced every three to four feet. This is termed subsurface injection. The feeding root area, in accordance with standard shade tree practice, was considered to be a doughnut-shaped area from the tips of the outermost branches inward about half way to the trunk.

The dose of chemotherapeutant was increased in direct proportion to the diameter of the trunk and was applied at the rate of five gallons of solution per diameter-inch at breast height.

b. Inoculation with *C. ulmi*

Trees have been inoculated by several methods in these studies, depending upon the objective sought in the particular experiment.

Method a. In one experiment conducted in 1945, inoculum was produced on agar plates on which were placed sterile chips of elm wood. On these coremiospores were produced. These were scraped from the agar surface and suspended in sterile water. Trees were then inoculated by selecting a centrally located twig about ¼ inch in diameter, bending this twig, placing a drop of spore suspension on it, and making a knife cut through the drop of inoculum. Under such circumstances, the drop of spore suspension was rapidly sucked into the woody tissue of the tree.

Method b. In studies involving street trees, it was undesirable to inoculate artificially. In such instances, plots were located insofar as possible in the midst of infected areas, and natural inoculation by bark beetles allowed to occur.

Method c. Subsequent to 1945, cultures of *C. ulmi* were grown in shake culture as described under the section on cultural studies. The very dense suspension of bud cells so produced in four days was diluted with an equal volume of sterile water to produce suspensions of about 1,000,000 bud cells per ml. In certain tests it was desired to inoculate trees as severely as possible to assure infection and to make the disease sufficiently severe that, if differences ascribable to chemotherapeutic treatment were evident, there could be little doubt of the effectiveness of treatment. In such cases inoculation consisted of chopping with an axe into the base of the trunk and pouring 10 to 25 ml. of such inoculum into the cut.

Method d. In other instances it was desired to simulate natural inoculation. A drop of inoculum from shake cultures, produced as described above and containing about 1,000,000 cells per ml., was placed in a twig crotch at least three years old and an incision was made into the wood through this drop. Two crotches were inoculated in this way on opposite sides of the tree. If the tree did not become infected, the procedure was repeated until six separate inoculations had been made at three times, each on two twig crotches.

c. Rating the effectiveness of treatments

Efficacy of treatment has been determined in all cases by estimating what proportion of the crown of a tree is involved with symptoms of Dutch elm disease. In some cases two operators have independently rated the same trees on the same day, in others a single operator has evaluated them, while in still other cases two operators have, in consultation, arrived at a mutual estimate of crown involvement. These estimates have been averaged over the plot and the averages are reported in the tables that follow.

3. Experimental

a. Relation of tree size to required dosage

At the outset, an important question was the relation of dosage of chemotherapeutant to size of tree on geometric considerations alone. That is, how much chemotherapeutant must be used to provide equal effectiveness on a large tree if the dosage for a small tree is established? This question must be answered independently of the relation between size of a tree and severity of disease.

Information on this point is best obtained from volume tables of wood content in relation to size of tree, and considerations of the tissues which are likely to become permeated with the chemotherapeutant.

For such purposes, the best volume tables are from German forest plots in which volumes include the small branches and twigs used for charcoal and firewood. Behm (6) has estimated the volumes for oak 150 years old, covering a wide range of diameter and height classes. Because elm is not a commercially used timber, volume tables in the detail required are not available. Wolfenbarger (82), with objectives similar to those under consideration here, has computed the volume of wood in American elms of various size classes. While very incomplete, his data can be compared with those given by Behm for oak to determine whether the two types of tree increase in volume with diameter in comparable fashion. A comparison of curves A and B in Figure 4 indicates that they do indeed behave similarly and that one is justified in examining how the dose of chemotherapeutant in oak should vary with volume, and applying these conclusions to American elm.

This question settled, one can proceed with the analysis. In the case of the healthy American elm, only the outermost annual rings function in translocating water and solutes to the leaves. The woody tissue more than three years old is heavily tylosed and, apart from xylem parenchyma and associated ray cells, which serve respectively in storage of carbohydrate and in translocation from phloem to the storage parenchyma, the other tissues of this age are dead and physiologically nonfunctional. Such tissue is likely to be largely nonfunctional insofar as a chemotherapeutant is concerned. It is accordingly neglected in these considerations.

The woody tissue with which we are here concerned has the shape of a tapered and branching hollow cylinder. It is the volume of this tissue which we wish to measure. Since we are concerned with relative volumes, the thickness of the walls of the cylinder are unimportant so long as they are uniform.

Summarized data from Behm's volume tables are presented in Table 5. They are selected by 10 cm. increments and are the median volumes for trees of all height classes having this diameter. Assuming that the shell constituting the functional cylinder in the tree has a thickness of 2 cm., one may calculate the volume of functional wood

(Column 4). This is the wood which must be permeated with chemotherapeutant. How the volume of functional wood changes with diameter may be determined by subtracting successive volumes of functional wood from the preceding value in Column 4. It will be seen that, for 10 cm. diameter increments, covering the diameter range of elms in the field, this relation is approximately a constant. It is shown graphically in Figure 4.

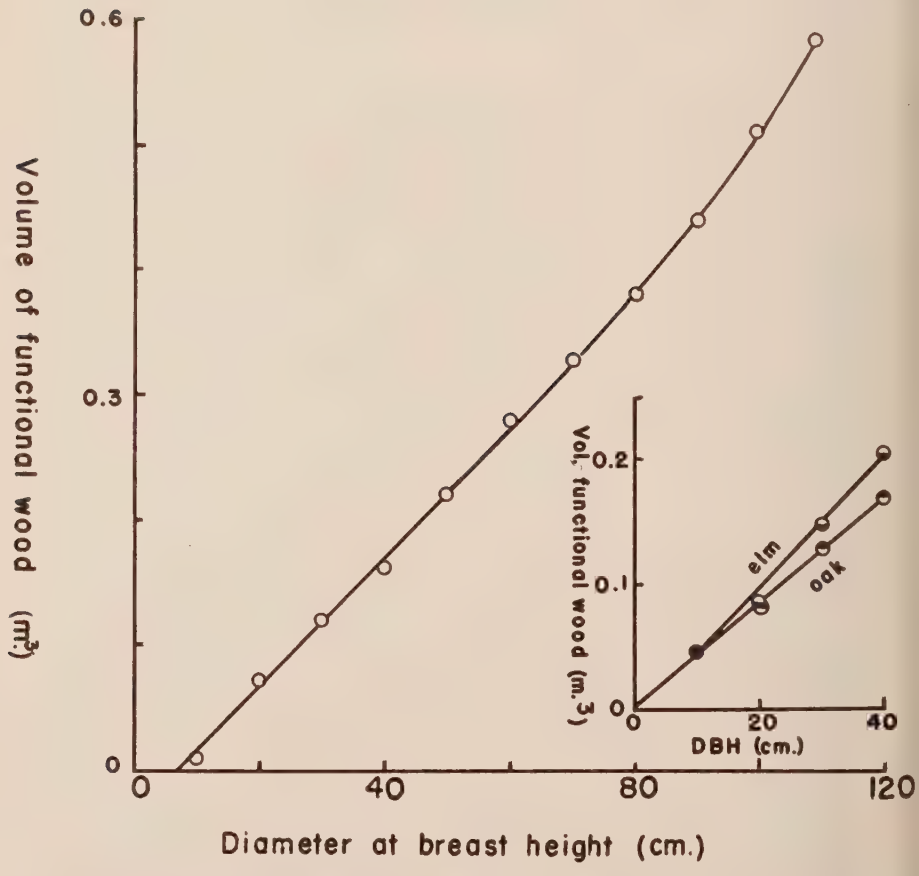


Figure 4. The relation between the diameter of a tree and the volume of functional wood. Data derived from Behm (6) for mature oak. Inset: Comparability between elm and oak for this relation. Data for elm derived from Wolfenbarger (48).

TABLE 5

Relation Between the Diameter of a Tree and the Volume of Functional Wood. Data Recomputed from Behm (6)

DBH[1]	*Volume all wood more than 3 cm. diameter*	*Volume of tree with diameter 2 cm. less than that stated*	*Volume functional wood*	*Relation of tree diameter to volume of functional wood*
cm.	m^3	m^3	m^3	m^3 *per 10 cm.*
10	.05	.04	.01	
20	.32	.25	.07	.06
30	.84	.72	.12	.05
40	1.63	1.47	.16	.04
50	2.69	2.47	.22	.06
60	4.03	3.75	.28	.06
70	5.69	5.36	.33	.05
80	7.68	7.30	.38	.05
90	10.01	9.57	.44	.06
100	12.71	12.20	.51	.07
110	16.13	15.55	.58	.07

The comparable relation for American elm, calculated from Wolfenbarger's data (82), is shown in Figure 4. It is apparent that the functional wood in American elm also increases in proportion to the diameter, within the limits of available data. While the slopes of the curves for oak and American elm are different, the critical factor is that they both present a linear relation of functional wood to tree diameter. These data are interpreted to indicate that, if the correct chemotherapeutic dosage is established for a tree of given size, it may be varied for trees of other size in proportion to diameter; in other words, chemotherapeutic dose is proportional to diameter of a tree.

In passing it should be noted, however, that there are grave dangers in establishing proper chemotherapeutic dosage on a small tree. A small error becomes multiplied many times in extrapolating from a whip-sized tree 1 cm. in diameter to one 50 cm. (approximately 20 inches) in diameter. In the case of an underestimate, this may lead to a grossly inadequate dose of chemotherapeutant.

b. The chemotherapeutic dosage

In 1945 Experiment A was undertaken at Mt. Carmel to determine the chemotherapeutic dosage of OQB on American elms (68). Plots of 10 trees, the trees averaging 1.5 inches DBH, were isolated by dykes to prevent solutions of chemotherapeutant from running out of the feeding root zone. Trees were inoculated as described under method a, above, on August 7, 1945, the day immediately following the last or preceding the first application of therapeutant. Treatments were applied on dates consistent with the timing and number of applications indicated in Table 6. The value of treatment was determined on August 28, 1945, in terms of per cent crown involvement by Dutch elm disease. On this same date, sample twigs were removed from portions showing symptoms (or points near where inoculation was made if no

[1]Diameter at breast height.

symptoms were evident). Chips from these were cultured in an attempt to recover the fungus. The experimental design and results are recorded in Table 6.

TABLE 6

Relation of Dosage, Timing and Number of Applications of OQB to Effectiveness of Treatment against Dutch Elm Disease

Conc. of solutions applied	*One application (10 gals./tree)*		*Five applications (2 gals./tree) on alternate days*	
	Pct. crown involvement	*Pct. of trees yielding C. ulmi*	*Pct. crown involvement*	*Pct. of trees yielding C. ulmi*
	Treatment before inoculation			
1:1000	6.1[1]	60[1]	4.5	70
1:2000	8.2	70	23.5	100
1:4000	19.8	70	10.5	70
1:8000	24.0	80	11.5	70
	Treatment after inoculation[2]			
1:1000	1.5	60	7.5	50
1:2000	9.5	70	9.5	90
1:4000	10.0	70	7.0	80
1:8000	12.5	90	5.5	80
Untreated checks	8.2	80		
	21.0	80		
	25.0	70		

[1]Each figure represents a plot of 10 trees.

[2]In this portion of the experiment, trees were inoculated on the day before the first application was made. No disease symptoms were evident when the treatment was begun. These data are, therefore, not comparable with those on curative treatments in the experiments which follow. In the latter case, curative effects were largely deduced from trees showing symptoms of disease when treatment was made.

Noting for the moment only the response of trees to which single applications of OQB were made, one sees a graded relation between concentration of chemotherapeutant and the amount of the tree which became involved with Dutch elm disease. This would indicate that concentrations of 1/1000 in water constitute more effective treatment than more dilute ones. Since such concentrations represent suspensions just beyond saturated solutions, higher concentrations have not been explored. Upon this basis, the standard concentration of 0.1 per cent OQB was adopted.

c. Number of applications

In Experiment A (Table 6) the chemotherapeutant was applied in two ways: (1) the entire dosage in a single application and (2) as a series of five applications made on alternate days. In both cases, the total amount of chemotherapeutant applied at the end of treatment was the same.

Comparing the relation of response to dosage in these two types of treatment, one is struck by the fact that while the response is graded for single applications, it is not for serial application. This result needs confirmation.

How may such an anomaly be rationalized? OQB and many another organic chemotherapeutant, is highly reactive under the proper conditions. In analytical chemistry, related hydroxyquinolines are used as reagents for detecting small amounts of such metallic ions as iron, copper, zinc and manganese. OQB almost surely reacts in the soil, probably being precipitated as a metallic complex. How much of the unreacted chemotherapeutant gets into the plant depends upon its concentration, that of the metallic ions existing in the soil solution, the pH of this soil solution, and soil temperature.

The amount of material reaching the plant also depends upon how much of the feeding root surface is in contact with the solution of chemotherapeutant. It might be inferred that a single application of 10 gallons per tree in this experiment thoroughly saturated the root surface and surrounding soil, whereas the dose of two gallons per tree did not. Likewise, at higher concentrations, the chemotherapeutant required longer to become inactivated in the soil and so was available to the plant for uptake for a longer time.

For the present, one may conclude that a single application at maximum dosage is more effective in practice than serial applications at fractional dosage (68). On this basis, subsequent experiments were designed to provide single applications at maximum dosage of OQB.

d. Recoverability of *C. ulmi* from treated and untreated trees

When attempts were made in Experiment A to isolate *C. ulmi* from inoculated trees after treatment, a high percentage of trees yielded positive cultures (Table 6), indicating that treatment does not eliminate the fungus to a major extent.

Moreover, on closer examination of the data, one observes that the percentage of trees yielding positive cultures is apparently related to the amount of involvement of the crown by Dutch elm disease. Figure 5, in which these are plotted against each other, strongly suggests such a relation. Check trees, being untreated, should form a different population, and have been ignored. If this relation exists, then dosage of therapeutant, which governs extent of invasion by the fungus, also governs the ease with which *C. ulmi* can be isolated from treated trees (68). Confirmation is needed, however.

To yield more information, Experiment B was designed, and it tested the relation under far more severe conditions and on a larger number of trees. A group of 191 elms, ranging from two to eight inches DBH[1], was divided into approximate halves. OQB was surface sprinkled on the soil around half of these trees on July 29, 1946. An equivalent amount of water was applied to the trees in the check plot. On the following day, all trees were inoculated with *C. ulmi* by method c. This method of inoculation is exceedingly severe, because it produces a basal infection which is known (2, 3, 4) to kill rapidly and quite uniformly, and from which the tree is unlikely to recover. Moreover, the quantity of inoculum used in each tree is vastly greater than

[1]Diameter at breast height.

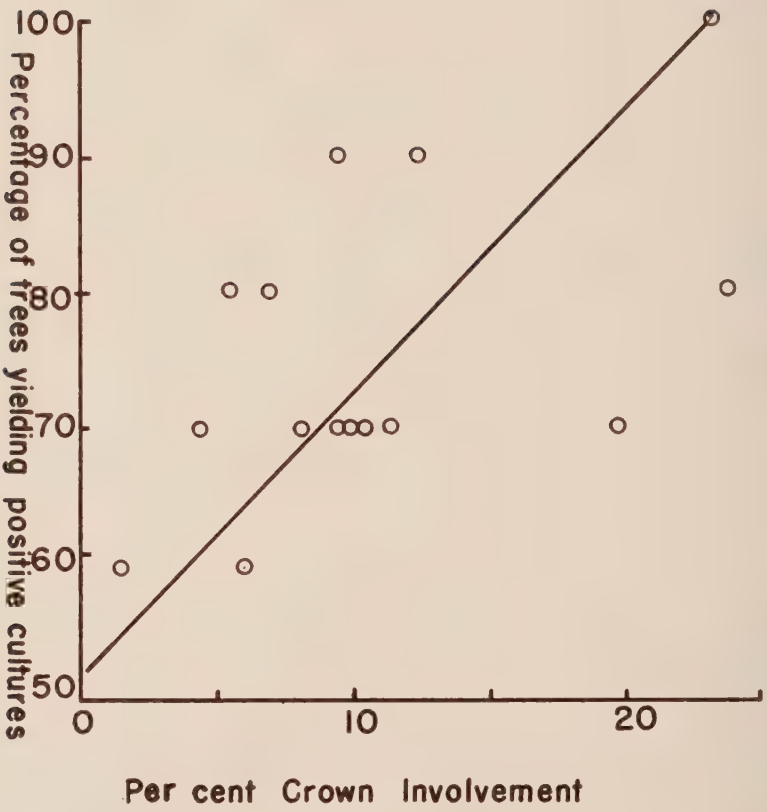


Figure 5. Relation between percentage of crown involvement by Dutch elm disease as influenced by chemotherapeutic treatment and ease of recoverability of *C. ulmi*.

a tree ever encounters in nature. Within ten days these trees showed severe symptoms of disease. During the last week of August, while symptoms of Dutch elm disease could be readily distinguished from autumnal drying and dropping of leaves, each tree was sampled and chips from them were cultured. The samples were taken from a portion of the tree showing symptoms of disease; where no symptoms were evident, they were taken from a comparable location. In cases where the fungus was not isolated, second and even third efforts were made to recover the fungus in culture. The results of these attempts are presented in Figure 6, together with the percentage of trees in the two plots which showed symptoms of disease. Here, again, it appears that the ease with which cultures may be recovered is related to treatment (18).

The ultimate fate of these elms, in light of the culture tests, is most illuminating. Active infections by *C. ulmi* ultimately developed in all check trees and all but one treated tree. The data presented in Figure 6, therefore, are not an indication that the fungus was not present when it was not recovered from a tree, but rather that treatment had reduced the vigor of the fungus so as to make its isolation more difficult.

These data also indicate that, in the majority of cases, the fungus can be isolated from treated and from untreated trees (18, 67). Treatment, therefore, does not kill out the fungus, at least at the levels of dosage and inoculum potential employed in these tests. Since culture filtrates are equally potent in the presence and absence of OQB, one is not justified in concluding that treatment brings about toxin inactivation in the tree. A more tenable explanation is that it is merely fungistatic: bear in mind that it was originally selected because of its ability to inhibit *C. ulmi* in culture (86). Whether the infection is completely stopped or not depends upon the levels of dosage and inoculum po-

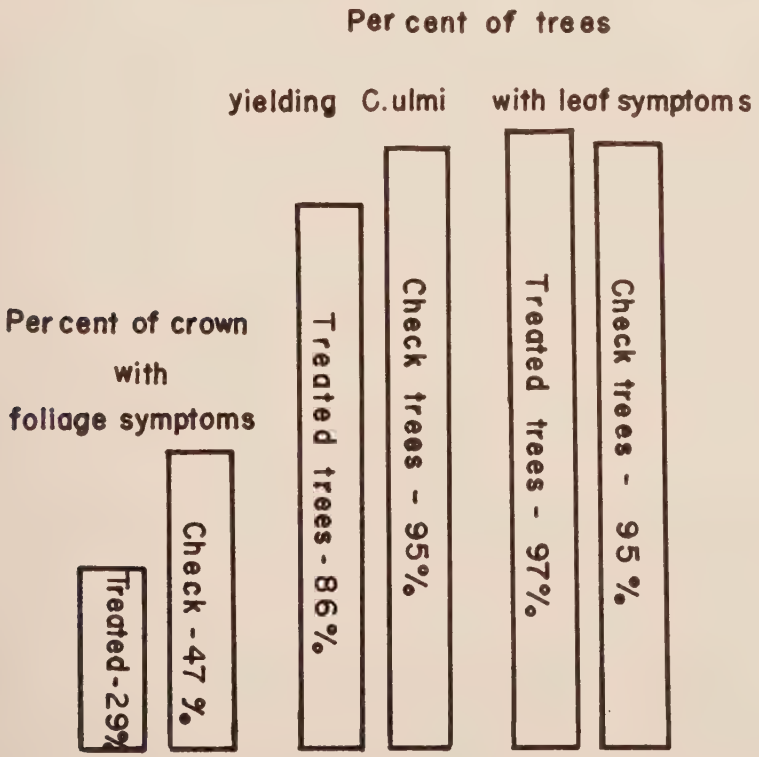


Figure 6. Relation between response of trees to treatment with 8-quinolinol benzoate and ease of recovery of *C. ulmi* in culture from them.

tential in a particular test (section j). Since OQB appears to be more fungistatic than fungicidal, it may well suppress growth of the fungus only when the dosage is adequate to be inhibitory.

e. Magnitude of curative (alleviative) effect

Data presented by Zentmyer *et al.* (86) and Stoddard (68) (see p. 29 above) had indicated that, on small trees and on occasional large ones, treatment with OQB suppressed symptoms of disease in some measure. To gain information on the magnitude of this effect, Experiment B, described on p. 31 above, was set up. On August 20, the trees were rated in the usual way and effects of treatment determined (18). At that time the portion of the plot which had been treated was less severely attacked by Dutch elm disease than the check portion. Thus, the percentage of the crown involved per tree in the treated portion was 29 per cent (average of 96 trees) and in the check portion was 47 per cent (average of 95 trees). This difference was very highly significant statistically. In the several other experiments reported in other sections of this bulletin, the alleviative effect of treatment is confirmed (See Tables 6, 7, 11 and 12).

Confirmation of this effect was sought on large diseased trees in varying stages of involvement. These were treated by the usual method. Such trees varied in size from 8 to 48 inches DBH and in involvement by Dutch elm disease from the earliest flags to 50 per cent or more of the crown. In many such cases, the disease advanced less rapidly after treatment than after none. In the vast majority of cases, however, such treated trees have died of Dutch elm disease. This experience has been amply demonstrated under practical conditions where tree experts have treated diseased trees. Such trees usually die, although they die more slowly than untreated trees do (21, 22).

Occasionally, however, trees having confirmed cases of Dutch elm disease recover. Whether recovery is more frequent among treated than untreated trees is not known, because large trees seldom recover from the disease. To determine whether such a difference is significant, more than a thousand samples of diseased trees among each group (treated and untreated) would have to be carried.

Evidently the value of curative treatment with OQB is related to size of tree and is somewhat less on large trees than on small ones (see sec. g, p. 36).

f. Life of a treatment

Information on how long the effect of a treatment lasts was obtained in Experiment B (see p. 31) by modifying its design somewhat in 1947. The check plot was left without further treatment. The plot treated in 1946 was divided into two subplots. On one of these, annual applications were repeated on May 15, 1947, and June 16, 1948. The other subplot received no further applications after 1946. Trees in these plots have been rated for involvement by Dutch elm

disease from time to time and effect of treatment has been evaluated. The averages for these plots on several dates are presented in Table 7.

TABLE 7

Value of Treatments and Duration of Effectiveness of OQB for Dutch Elm Diseased Trees

Treatment	*Averaged percentage of crown involvement by Dutch elm disease*				
	8/20/46	*7/2/47*	*8/29/47*	*6/16/48*	*8/6/48*
Each year	29	26	35	33	33
1946 only		42	51	50	49
Untreated	47	44	53	48	49

Clearly, the trees in the plot treated in 1946 only degraded to the level of the check trees by midsummer of 1947, whereas trees on which treatment was repeated in 1947 maintained their level of protection. This difference has been maintained through 1948.

Evidently treatment must be repeated each year in order to maintain the protection, but it need be made only once a year for this purpose. Whether more frequent treatment will reduce the amount of crown injury by Dutch elm disease is not indicated by this experiment. Another experiment was designed to provide evidence on this point. The plot consisted of large trees which could not be inoculated artificially and none of them have become infected naturally. As yet, no evidence on this point has been obtained.

The data in Table 7 (Experiment B) point to two interesting conclusions. In 1947, trees in the treated and untreated plots degraded from disease about 9 per cent during the growing season. Under inadequate treatment, trees degrade to the level of check plots and to do so must deteriorate more rapidly than check trees. That those in the annually treated plots did not do this is ascribed to the effect of treatment.

In 1948 none of the three plots increased in disease level during the growing season. This is ascribed to the fact that the trees are not very large, and that those not killed by Dutch elm disease in the winter of 1947-48 are all outgrowing infection and will probably survive. By late summer in 1948 there were almost no signs of active Dutch elm disease in the plot. If this reasoning is correct, treatment has been sufficiently effective to save 16 per cent more trees than would have survived without treatment. However, this difference between treated and untreated plots was apparent in 1947 and added treatments have not further increased it.

The effect of treatment indicated by the percentage of trees surviving infection is more striking. These data are presented in Table 8.

TABLE 8

Effect of Treatment with OQB on the Number of Trees Surviving Dutch Elm Disease

Treatment	*Percentage of trees originally treated alive:*	
	in 1947	*in 1948*
Each year	94	74
1946 only	60	55
Untreated	68	57

g. Relation of tree size to response

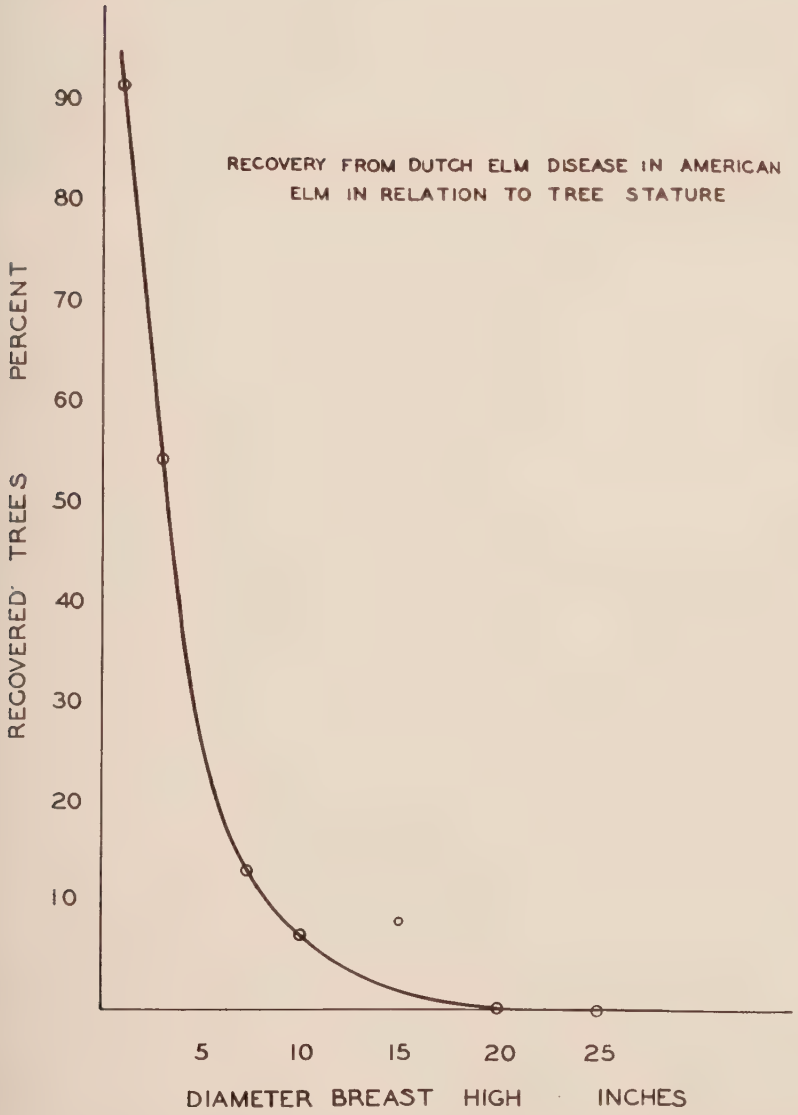
(1) RELATION OF TREE SIZE TO SEVERITY OF DISEASE IN THE ABSENCE OF TREATMENT

The relation between the size of a tree and the dosage of chemotherapeutant was discussed on page 27 in terms of the way in which the volume of wood to be protected varies with diameter. If this factor alone governs the amount of protection which a tree receives as a result of treatment, there should be no relation between size of a tree and response to treatment, so long as dosage of chemotherapeutant is increased in proportion to size. If, however, there is a relation between the size of a tree and the severity of Dutch elm disease, one may expect a relation between size of a tree and its response to treatment.

Excellent data on this relationship (i.e., between size of a tree and severity of Dutch elm disease) have been obtained by Banfield, Rex and May (5). The criterion used in these studies was whether the disease recurred in a tree after the first year of infection, and this factor was related to diameter of the tree (Figure 7).[1] The trees in this study were all naturally infected and were not treated in any way. Here, it may be readily seen that the disease tends not to recur in very small trees, but to do so more frequently the larger a tree is. There is, then, a very definite relation between size of a tree and recurrence of disease in ensuing years. Usually the disease recurs until the tree dies (5).

The criterion used is but one of several which measure severity of disease. Severity of disease may also be measured by the percentage of the crown which becomes involved in a stated period of time. This is the criterion which has been used in the chemotherapeutic studies. It depends in magnitude upon many factors, two of the most important ones being where infection occurs and the extent of vascular invasion of the tree by disease when measurements are begun. Banfield (3) has studied the relation between extent of vascular invasion and recurrence of disease, and noted that basal infections or those involving the central bole of the tree are most likely to recur in following years. The percentage of crown involvement by disease will also be greatest when infections involving the central bole occur.

[1]We acknowledge with thanks permission by Dr. Banfield and the Division of Forest Pathology, USDA, to publish the curve in Figure 7.



Fiture 7. Relation of tree diameter to incidence of recovery from Dutch elm disease. This figure and data were obtained by Banfield, Rex and May (5) and are used through their courtesy and that of the Division of Forest Pathology, U. S. D. A.

The trees in Experiment B were inoculated by method c, which consisted of chopping into the trunk with an axe and pouring a spore suspension of *C. ulmi* into the cut. Such infections are very likely to recur and data based upon them will not exactly follow the curve of Figure 7 because recurrence is more likely than under conditions of natural inoculation. However, a similar relation of size of tree to severity of disease will hold. In a plot in which all trees were inoculated similarly, severity of disease may be measured soundly in terms of the percentage of crown involvement, or of the percentage of trees remaining alive by the end of the season.

(2) RELATION OF TREATMENT TO THE SIZE OF TREES SURVIVING DISEASE

Having at hand the relation shown in Figure 7, we may compare the relation between tree size and severity of disease for two populations of trees: those treated chemotherapeutically and those not treated. If chemotherapeutic treatment is an effective curative measure, it may be that the trees surviving infection in any one year will be larger if they are treated than if they are not.

In Experiment B, data were taken so as to yield information on this point. On August 29, 1947, all trees were calipered to the nearest tenth of an inch, and tree size was correlated with response to chemotherapeutic treatment. Table 9 summarizes these results by treatments.

TABLE 9

Relation Between Tree Diameter and Effectiveness of Chemotherapeutic Treatment

Treatment	*Pct. of trees remaining alive*	*Average diameter of trees*	
		alive	*dead*
Each year	94	2.16	2.97
1946 only	60	1.94	3.29
Check	68	1.76	2.20

The average diameter of trees in the check plot was 1.88 inches. A comparison of the size of trees remaining alive with those dying in 1947 reveals that statistically the larger trees died and the smaller ones survived. Clearly the relation indicated in Figure 7 applied qualitatively to the present study.

If there were no effect of curative treatment by chemotherapy, then the diameter of trees in the treated plot remaining alive should be the same as the corresponding figure in the check plot. This is clearly not the case. As treatment became more effective, through proper timing of treatment, the size of trees surviving disease increased. Evidently proper treatment enables larger trees to survive the disease. This effect will serve as an objective measure of the

effectiveness of any given chemotherapeutant or of techniques of application under field conditions.

It will be noted, however, that the trees which died despite treatment were small by practical standards. Trees larger than three inches in diameter must be saved by curative treatment if the method is to have practical value. This may become possible with better chemotherapeutants or with better techniques of application. We have already seen (page 18) that treatments applied to a diseased tree will, in general, be applied after the disease has caused irreversible changes. Treatment will not alter this fact. Under these conditions, distribution of the chemotherapeutant to the points needed for preventing further blocking of the vascular system by gums and tyloses will be much less perfect than if the chemotherapeutant is applied to the healthy tree to prevent disease. The chances are, then, that curative treatments will be less perfect than preventive ones, in light of this reasoning.

(3) RELATION BETWEEN TREE SIZE AND SEVERITY OF DISEASE IN TREES UNDER TREATMENT

Another way of measuring effectiveness of treatment is to relate severity of disease as measured by percentage of crown involvement to size of the tree between populations which have received treatment and those which have received none. To do this, data from Experiment B were assembled in terms of severity of disease on trees in size classes which increased by 1 inch increments. This was done for each plot separately. The resulting relations have been plotted in Figure 8, where tree diameter is plotted against percentage of crown involvement by Dutch elm disease. The regression is upward with tree diameter in all cases and it may be clearly seen that small trees suffered less from disease than large ones. For small trees, Curves A and B (treated plots) lie considerably below Curve C (check plot), which indicates that treatment resulted in less damage to the tree. The three curves merge and become essentially identical for large trees (four inches and over), which indicates that these were not benefited by treatment. It should be pointed out, however, that the majority of trees in these plots were four inches or less in diameter. Hence, the relation shown in Figure 8, is most reliably determined in the region where differences exist.

How these trees behaved in 1948 as compared with 1947 is of interest in this connection. For purposes of brevity, the net change in percentage of crown involvement in 1948 as compared with 1947 has been calculated and this has been related to tree diameter (Table 10).

These data indicate that disease indexes remained about the same or decreased on small trees, as the trees outgrew the disease, but increased sharply on larger trees that had not already died. They further indicate that curative treatment, at least with 8-quinolinol benzoate, is insufficient to hold Dutch elm disease indefinitely.

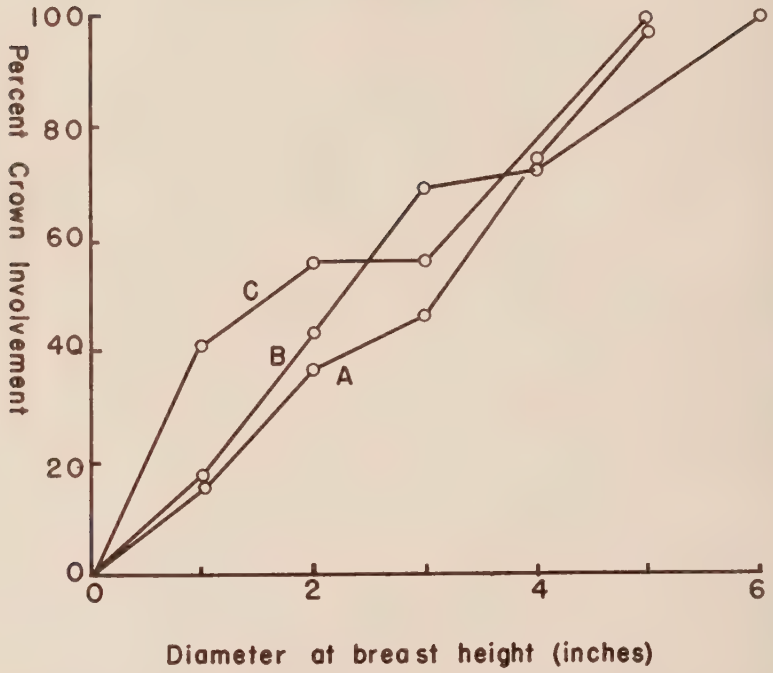


Figure 8. Relation between size of tree and response to treatment with 8-quinolinol benzoate as measured by percentage of crown involvement. Trees in Curve A were treated each year; those in Curve B treated in 1946 only, and those in Curve C were not treated.

TABLE 10

Change in Crown Involvement Between 1947 and 1948 as Related to Tree Diameter and Chemotherapeutic Treatment

Treatment	*Change in percentage crown involvement from 1947 to 1948*						
	Tree diameter (inches)						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Each year	—5	—21	+10	+25	—3	+20	+35
1946 only	—6	—4	—1	+25	+30	0	0
Check	—4	—2	—15	0	0		+2

(4) SUMMARY

In conclusion, let us refer back to page 29, which deals with the relation of increasing size of tree to dose of chemotherapeutant. It was pointed out there that a small underestimate on small trees in what constitutes the chemotherapeutic dose will be very greatly multiplied

in extrapolating to find the proper dose for a large one. Figure 8 indicates that as trees become larger, the protection is less. Evidence by size classes is insufficient to indicate whether the effect noted here is caused by an error in estimating the chemotherapeutic dose or by the relation between tree size and severity of disease apart from chemotherapeutic treatment. From other evidence (p. 46), this effect may be ascribed to the relation of tree size to severity of disease.

The observed behavior indicates that it is exceedingly dangerous to draw conclusions on the behavior of large trees from the response of small ones. If one is working well within the limits of phytotoxicity, it is evidently desirable to double or triple the chemotherapeutic dose determined by tests with small trees, before attempting application on large ones.

The evidence from controlled tests reported here will indicate why experience has been so disappointing when OQB has been applied to diseased trees in the hope of curing them under practical field conditions.

h. Method of application

(1) SOIL APPLICATION

To find how the method of applying a chemotherapeutant to the soil affects the degree of protection Experiment C was set up. The same amount of OQB was applied to three plots consisting of 25 trees each in the following ways: One plot was treated by scattering the dry powdered OQB over the surface of the soil and then watering it in with a volume of water equivalent to standard treatment. Another plot was surface sprinkled with OQB in aqueous solution. The third plot received subsurface injection, as described under methods of application, (page 25). The check plot received water alone. All plots were treated on May 20, 1947.

The trees were inoculated by method d (p. 26). Each tree was inoculated in two locations on opposite sides of the trunk on May 26. Trees showing no symptoms of disease by June 15 were reinoculated June 17 in similar manner. Readings of disease were again made on July 7 and those still showing no symptoms were again reinoculated on July 18. Thus, some trees received two, some four and some six inoculations on one, two and three dates, respectively. Trees were rated in the usual way, and the resulting data are assembled in Table 11. In this test subsurface injection was best, surface sprinkling second and dry scattering followed by watering was of no value.

It is likely that differences between subsurface injection and surface sprinkling treatments would be greater than those recorded in Table 8, had the comparison been made on trees growing openly. The trees in these plots range from one to seven inches DBH. They were overgrown and crowded in an elm nursery, and their root systems were very shallow. Under such circumstances the differences between these methods of application would not be so great as on deeper rooted trees.

TABLE 11

Preventive and Curative Value of OQB Against Dutch Elm Disease as Determined by Method of Soil Application

Method of application	*Per cent of crown involved*			
	7/7/47	*7/29/47*	*8/14/47*	*6/14/48*
Dry broadcast and water	22	35	58	68
Surface sprinkle	7	15	41	42
Subsurface injection	0.6	6	31	45
Checks	19	29	50	65

(2) TRUNK INJECTION

Zentmyer *et al.* (86) used trunk injection for the major part of their work. Stoddard (69) has used top injections for introducing chemotherapeutants into peach trees in studies on X disease. When introduced by these means, distribution of the chemotherapeutant is inferior to that obtained with soil application. The structure and properties of wood are such that it offers very great resistance to flow in radial and tangential directions as compared with longitudinal. Any injection method will suffer from this fact, but radial distribution of chemotherapeutant is unnecessary as long as peripheral and longitudinal transport occur. Peripheral distribution can be improved by introducing the solution through many holes, distributed around the circumference of the tree. This causes extensive wounding, particularly when treatment must be repeated periodically.

As a result of poor distribution in the tree, the response of plants treated by injection has proven erratic. Thus, in chemotherapy studies on Fusarium wilt on tomato, the response is far more varied when a chemical is hypodermically injected into the vascular system than when it is applied to the soil.

Despite all of this, some technique must be devised to treat trees which are surrounded by pavement on city streets. Trunk injection or foliage spraying constitute the only ready means of introducing the chemotherapeutant into the tree. To date, evidence would indicate that there is greater promise from trunk injection than from absorption of a therapeutant through the foliage. Because of these considerations, Experiment D was set up in which an effort was made to see how effective trunk injection is as compared with soil application. One-tenth per cent solutions of OQB, using isopropyl alcohol as a cosolvent were injected into trees in this case. The test and check plots each consisted of 25 trees, ranging from two to five inches DBH

An injector comparable with the one described by Southwick (65) was employed. A hole was bored into the trunk of the tree and a ½ inch galvanized close nipple with lateral holes bored in it was screwed into this hole. Bleed valves were installed just outside this,

and the apparatus was then connected with pressure hose to a heavy tank containing the solution. This was in turn connected to a portable air compressor which supplied the driving pressure for injection. In operation, air was exhausted from the line before injection of the tree was begun.

In injecting a tree, holes were spaced every four to six inches around the circumference. Because trees varied very widely in rate of uptake and because uptake varied exceedingly from day to day with meteorological conditions, no effort was made to inject the same quantity of material into each hole. This would have been impossible. They were treated June 18 to 20, 1947.

These trees were inoculated just before treatment on June 17 and those not showing infection within three weeks were reinoculated July 18, 1947. These plots are roughly comparable with those in Experiment C, being located in the same nursery. Because they were inoculated fewer times and later in the season, other things being equal, one would expect greater differences between treated and untreated injected trees in this experiment than in the trees in Experiment C. This, however, is not the case. The order of protection was considerably less and is recorded in Table 12.

TABLE 12

Value of Trunk Injection by OQB in Combating Dutch elm disease

Treatment	*Pct. crown involvement*		
	7/29/47	*8/14/47*	*6/14/48*
Trunk injected	13	28	49
Check	18	27	67

Thus, while trunk injection reduces slightly the severity of Dutch elm disease over controls, it is less effective than soil application as a method of treatment, and will require much better chemotherapeutants than OQB before its value can be explored on a practical basis.

i. Preventive action

While no one would deny that curing a sick elm of Dutch elm disease would be an ultimate goal in such work, the fact remains that learning how to prevent the disease among healthy trees represents a long step forward over past facilities for dealing with it. No chemotherapeutic treatment which significantly prevents infection can be considered inadequate, however poor it may be in curing sick trees.

One is led to wonder if the difference between prevention and therapy of early infections is not in large measure a matter of quantity of pathogen to be overcome by a chemotherapeutant: the inoculum potential. In the case of an old infection, there is a qualitative difference as well, because the pathogen produces irreversible changes in the host. In elm this takes the form of tyloses, gum plugs, and

attendant resistance to water movement in the xylem. It appears as wilted leaves (which wilt may or may not be reversible depending upon whether it is of physiological or pathological type (13) and as defoliated branches, and finally as dead buds and branches. But in the very young infection, before such irreversible changes have occurred in the host, the difference between therapy and prevention may be one of time, and quantity of inoculum.

Concurrently with experiments testing the curative value of OQB, experiments were set up to examine preventive value of treatment. Experiments C and D described in the two previous sections have yielded information on preventive value of treatment. Treatment was applied either a week after inoculation (Experiment D) or almost simultaneously with it (Experiment C) and inoculation was by method d, designed to simulate natural inoculation in some measure. Trees were inoculated repeatedly when they failed to become infected after the first inoculation. The relation between type of treatment and number of trees remaining healthy is reported in Table 13.

TABLE 13

Relation Between Type of Treatment and Number of Trees Remaining Healthy

Method of application	*Pct. trees remaining healthy*[1]			
	7/7/47	*7/29/47*	*8/14/47*	*6/14/48*
Experiment C				
Dry broadcast and water	36	25	0	1
Surface sprinkle	31	19	10	1
Subsurface injection	58	32	10	2
Checks	33	21	5	0
Experiment D				
Trunk injected		45	24	8
Check		24	16	4

[1]All trees remaining healthy on date noted were reinoculated as noted on p. 42.

It is of interest to note that the relation between method of treatment and the number of trees remaining healthy despite inoculation rates the methods of treatment in the same order as the ratings in terms of percentage of crown involvement by Dutch elm disease.

Data presented in Table 13 would indicate that treatment by OQB causes increased resistance to infection by *C. ulmi*; it appears to prevent infection. This evidence is supported by another experiment (Experiment E) to be discussed later (p. 61) in which newly transplanted elms from $2\frac{1}{2}$ to 3 inches DBH were divided into several groups and preventive applications of OQB applied to certain groups and not to others. None of the trees in the groups to which OQB was applied became infected naturally. Three trees not so treated became infected. There were 53 trees in each group under treatment.

The effect of OQB as a curative treatment has been shown to be much smaller on large elms than on small, nursery sized trees. One

would expect that when OQB is used as a preventive measure, the same relation will hold: large trees will not respond to treatment as well as small ones do. To check on this point, some experiments on such large trees are of interest. In collaboration with the Park Department of the City of New Haven, an experiment (Experiment F) was established on the New Haven Green. In 1946 the first trees became infected with Dutch elm disease there. They were immediately removed. Trees on the lower green were divided into three lots: those receiving no treatment, those receiving surface sprinklings of OQB and those receiving subsurface injections. None of the trees on the upper green was treated. In all, there were 152 trees receiving no treatment, 15 receiving subsurface applications and 38 receiving surface sprinkling. The average diameter of all trees was 16 inches, and they all received periodic sprayings against leaf eating insects. As additional trees have become diseased, they have been removed. OQB was applied first on August 8 to 12, 1946, and was reapplied on May 19, 1947. In 1948 application was made on May 12. Such trees as have become diseased did so naturally.

Since this test has been designed as a preventive treatment, the data have been recorded as numbers of trees becoming infected, rather than amount of involvement per tree. This information is recorded in Table 14.

TABLE 14

Preventive Action of Oxyquinoline Benzoate Against Dutch Elm Disease

Year of treatment and observation	*Number of trees becoming infected with Dutch elm disease in year noted*	
	Treated	*Untreated*
1946	0	0
1947	0	8
1948	3	15
Total	3/53	23/152
Pct. becoming diseased	5%	15%

These data confirm on large trees the observations made on small ones: that OQB does exert preventive action against Dutch elm disease, if applied to healthy trees. Other observations will also bear this out.

The City of Waterbury began a program of OQB treatment on their Green in the fall of 1946. Two applications were made in 1947 and treatment was continued in 1948. Of 52 trees, 10 were infected in the spring of 1947, but in 1948, only one additional tree became infected. It is true that there is no check plot in this case and one cannot say if the disease had become less severe in 1948; such an occurrence is improbable in this location, however.

Another bit of evidence may be drawn from a practical operation, carried out in Hartford. On two adjacent blocks there are the landscaped grounds of two large companies. Starting in 1947 and again in 1948 OQB was applied to 17 of the larger and more valued trees

on one of these. Trees on the other grounds were not treated and constituted a check plot on the operation, together with adjacent street trees. By late summer of 1948, only one tree among those treated had become infected, whereas eight trees among those not treated became diseased. All of these point to the conclusion that treatment protects the tree against infection to a considerable degree. Protection is no more absolute than it is against any disease, however.

So far as we can measure it at present, it appears that the magnitude of the preventive effect of OQB is independent of size of the tree, if the dosage of chemotherapeutant is increased in proportion to diameter of the trunk. On page 28 we concluded from geometrical considerations alone that amount of chemotherapeutant should be increased proportionately with diameter of the tree to maintain a uniform dosage in trees of varying size. Evidently this conclusion is approximately correct, as are the underlying assumptions regarding distribution of the chemotherapeutant.

When OQB was used as a *curative* treatment, however, it was readily demonstrated (pages 40 and 41) that the curative effect became less as size of the tree increased, even though amount of therapeutant was increased as indicated. In this case the primary responsible factor was surely the greater severity of disease on large trees than on small ones, a factor which is independent of treatment (see Figure 7).

j. Effect of inoculum potential on protective action

Data on this point are meagre and are drawn from practical experience over the State, as observed by tree experts. It would appear that protection is greatest where incidence of bark beetles and Dutch elm disease is least. This would be expected. In spraying potatoes for late blight or apples for scab, one expects reasonable control of disease under ordinary years when inoculum potential is moderate. In years when an epiphytotic develops, the level of control drops if the same program of spraying is used in both circumstances.

Apparently in those areas where Dutch elm disease has existed for the longest time, and where the number of diseased trees and bark beetles is highest, the percentage of trees coming down with Dutch elm disease despite treatment is also highest.

k. Timing of applications

(1) WITH RESPECT TO INOCULATION

The factors involved here have already been discussed. It would appear that whether treatment is made before or after inoculation is a matter of quantity rather than quality of inoculum potential. As discussed above, when inoculum potential is low, protection is highest.

(2) WITH RESPECT TO SEASON

Granted that treatments are most effective if applied to be prophylactic, there is still the question as to proper timing of applications

for maximum effectiveness. If treatment is designed to be preventive, one might wish to apply it just prior to or coincident with the peak of emergence of the elm bark beetle. In the experiments reported above, this has been done to a large extent. Applications have been made in mid-May to early June. In 1947 a few trees were treated in April, all of them being diseased. These, in contrast with similarly diseased trees treated in May after foliage buds were unfolding, showed no response whatsoever to treatment. Probably if applied before heavy water intake by the tree occurs in the spring, OQB is rendered inactive in the soil before it can be absorbed by the tree in any quantity.

Logically, applications made in mid-May and in mid-July ought to be the most effective. This is when protection is most desired. Treatments so timed are the ones which have produced greatest effects in the experiments reported above.

4. Conclusions

The field experiments with OQB have suffered from experimental design. Soil applications of OQB to small trees planted in established nursery rows required treatment of blocks of trees rather than treatment of single trees randomized through the planting. This can be avoided only by plantings especially made for such work. With a slow-growing crop such as elms, such practices have not been feasible until the recent successes in chemotherapy have made long range planning in this field desirable. Likewise, tests conducted on large street trees have suffered from design, because large trees, growing under comparable conditions, cannot so readily be treated in quantity and at random as smaller plants can. Despite this severe limitation, certain conclusions stand out clearly from the studies reported.

1. Studies with OQB were undertaken in an effort to find the answer to two problems: (a) what are the limitations of extrapolating results obtained on small trees to large ones and (b) how valuable is oxyquinoline benzoate as a chemotherapeutant under practical conditions.

2. The dosage of chemotherapeutant should increase with size of tree in direct proportion to diameter at breast height. This assumes that the chemotherapeutant becomes distributed only through the conductive sapwood and leaves. Errors in the chemotherapeutic dose based on small trees will be greatly exaggerated in extrapolating this dose to apply to large ones.

3. Based on dosage-response relations for American elm suffering from Dutch elm disease to OQB applied to the soil, the chemotherapeutic dose has been established at 1:1000 concentrations applied at the rate of five gallons per diameter inch at breast height.

4. One application at this rate gave greater response than five applications on alternate days at one-fifth of this rate.

5. *C. ulmi* can be recovered in culture from treated and from check trees with equal frequency. This may mean that OQB is not

eradicative in the tree or that the chemotherapeutic dosage has been underestimated.

6. OQB can be demonstrated on small trees to exert an alleviating action on diseased trees. They do not suffer so badly from disease as untreated ones do and, consequently, die more slowly. However, diseased trees usually die despite treatment, if they are large, and small ones frequently recover from disease despite no treatment.

7. Treatments must be reapplied each year. Whether applications of greater frequency will increase effectiveness to a practical degree is not yet known. But available evidence indicates that OQB is fungistatic and that when the concentration in the tree drops below the fungistatic dose, the fungus will burst into activity again.

8. The larger a tree is, the more it suffers from Dutch elm disease. The dosage of chemotherapeutant should be increased in proportion to diameter for constant level of chemotherapeutant in the tree. These two factors, acting differently, may indicate that dosage of chemotherapeutant should be increased at a greater rate than the geometry of the tree would indicate, since a small infection in a large tree is so much more damaging than a similar infection in a small tree. It becomes essential to keep the small infection from becoming larger in a big tree. This is more likely to be accomplished at a high chemotherapeutic dosage than at one adequate for a small tree. If this be true, there must be a wide margin of safety between the chemotherapeutic dosage and the phytotoxic dosage.

9. Soil applications of chemotherapeutant have given more uniform and greater responses than trunk injection. Foliage applications of chemotherapeutants have not yet been examined. Greatest success has attended applications applied beneath the surface of the soil in the feeding root area.

10. Preventive applications of oxyquinoline benzoate appear to give protection against infection of practical magnitude on both small and large trees. However, it has been adequately shown that oxyquinoline benzoate suppresses symptoms of disease, especially in early stages. It is possible that the observed differences reflect symptom suppression rather than true prevention of inoculation. If this be true, however, trees in treated plots, in the second and following years of treatment, should become infected at the same rate as untreated plots. This is apparently not the case. (See Table 14.)

11. Preventive applications appear to be most effective when the incidence of disease about the treated trees is low, and effectiveness appears to decrease as disease incidence in the community increases.

12. Applications timed to precede emergence of bark beetles slightly or to coincide with the peak of emergence appear to be more effective than applications timed greatly before or after this event. This could be expected from a knowledge that the chemotherapeutant has a relatively short life in the soil or in the tree, and must be active when inoculation occurs.

III. Spray Experiments For Control of *Scolytus multistriatus* Marsh.

A. INTRODUCTION

In this country Dutch elm disease is spread chiefly by the smaller European bark beetle, *Scolytus multistriatus* Marsh. This beetle constructs its brood galleries under the thick bark of the trunks and large branches of elms which are in a sickly condition. Frequently, the cause of this condition is Dutch elm disease. Beetles which emerge from such diseased trees may carry spores of the fungus *C. ulmi* on their bodies. These beetles feed in the crotches of small elm twigs and, if the feeding puncture penetrates to the vessels, the spores gain entrance to the vascular system of the tree. There the spores may germinate, and so may infect a healthy elm tree with this disease.

There are two annual broods of bark beetles. The adults of the first brood usually begin to appear at about the time when the elm leaves are half developed, generally during the latter part of May. The emergence of beetles of the early brood continues throughout the early summer, passing through a period of peak emergence when they are most abundant. Beetles of this brood may still be emerging when the adults of the second generation are just appearing, about the middle of July. The beetles of the latter generation pass through a peak abundance, and continue emerging until late in September.

The use of an insecticide to protect the twigs against bark beetle feeding would at once appear to be the obvious preventive measure. Shade trees commonly are protected from attack by many other insects, chiefly defoliators, by this method. In the case of defoliators, however, absolute prevention of feeding is not necessary, nor is it expected. If one of the so-called "stomach" poisons is used, the insect must ingest sufficient leaf tissue to obtain a lethal dose of the poison. The loss of a small percentage of foliage area from a large tree will not be noticed, nor will this loss seriously affect the tree.

Whatever the insecticide used, many of the common shade tree pests can be killed with a comparatively light dosage, particularly if the timing of application is correct. Furthermore, most of the individuals of a given population of one of the defoliators appear at approximately the same time, and one spray application is adequate. The insecticide need not retain its toxicity over a long period.

In contrast, spraying for control of elm bark beetles poses distinct and more difficult problems. It is obvious that the beetles should be killed or inactivated by the insecticide before they can feed. It would make little difference whether or not the beetles eventually died, if they first were able to feed, and perhaps to inoculate a healthy tree. The insecticide must also retain a long-lived residual toxicity, as the emergence of adult beetles of either of the annual broods may take place over a period of several months.

None of the standard insecticides, in use for many years, possess the qualities outlined above. European workers attempted control measures with some of these, but judged them to be unsuccessful. Fransen (28, 29) and Roepke (57) thought that lead arsenate and Paris green might exert a fungicidal action, although they did not prevent beetle attack. Roepke even stated that young elms sprayed with these materials were attacked more readily than unsprayed trees. Fransen (30) found that rotenone dust would kill all beetles treated with this material within 24 hours.

In this country, Felt (25) and Bromley (26, 27), D. L. Collins (15, 16), C. W. Collins et al. (14), and others (50, 58) have used various materials, including arsenicals, but the results cannot be considered satisfactory. Wilcoxon and Hartzell (79) employed *S. multistriatus*, among others, as a test insect in experimental work on certain organic thiocyanates, with but moderate degree of mortality.

Most of the experiments by all of these workers were made with small, potted elms. The test beetles were confined to these by means of cages. D. L. Collins (15) placed such potted trees in a large cage, where the beetles had a free choice of feeding material. He found that variations in the amount of feeding occurred irrespective of the sprays (containing lead arsenate, in varying dosages), and seemed to depend more on the relative positions of the trees in the cage. He states: "Although the data on these experiments have not been properly evaluated, the significant point to be made here is that a heavy coating of spray on many trees does not always lessen the amount or severity of feeding".

It is apparent that these insecticides are not satisfactory for elm bark beetle control. The so-called "stomach" poisons fail initially because they do not take effect fast enough to prevent feeding. The contact insecticides then available lost their residual toxicity relatively rapidly on exposure to atmospheric conditions.

It was not until the long-lasting insecticidal properties of DDT residues were recently discovered that control of elm bark beetles came within the realm of possibility. Whitten and Parker (78), as a result of preliminary trials, first suggested that DDT sprays might be used for control of these beetles. Since that time, other synthetic organic insecticides have become available. Some of these possess properties which automatically preclude their use on shade trees. Others may eventually prove to be even more satisfactory than DDT for this purpose.

Once an insecticide possessing the necessary qualities is at hand, there still remain the problems of dosage, distribution and formulation. The majority of the small twig crotches in which the elm bark beetles feed are found in the upper part of the tree crown (82). Thus, that portion of the tree most difficult to spray with ground equipment must be protected. It must receive a dosage which will inactivate beetle adults throughout the feeding period. The insecticide must be distributed uniformly throughout the crown, and an attempt must

be made to cover all vulnerable twigs. Theoretically, one infected beetle might inoculate a tree with but a single feeding puncture, although this probably seldom happens. Formulation of the insecticide is important because of the high concentrations and heavy dosages which must be employed. Used at ordinary dilutions, many formulations are quite safe on elm trees, and on other plants as well. However, burning and killing of plant tissues may result when the same materials are used in more concentrated form.

These are some of the factors which differentiate insecticidal control of elm bark beetles from that of other shade tree pests. In addition, there are other considerations. The restrictions imposed by the location of elm trees in urban communities must also be taken into account. Such trees frequently are so located that it is difficult to treat them properly and at the same time avoid spraying near-by houses.

B. EXPERIMENTS IN 1947

In 1947 investigations were initiated at this Station to determine whether or not elm trees may be protected against infection by Dutch elm disease by means of sprays directed against the smaller European bark beetle. Certain phases of this work were conducted in cooperation with the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The experimental spraying was done only with the mist blower, as hydraulic methods were being tested elsewhere. The blower used was a converted Bean "vapo-duster", described by Potts and Friend (54) and has a capacity of 8,000 cubic feet of air per minute.

A section of Edgewood Avenue, New Haven, in an area of high disease incidence, was selected as an experimental plot. This plot contained a total of 86 trees, averaging 62 feet in height. It was intended that the trees would be sprayed while in the dormant stage, but inclement weather forced postponement of the operation until late May. The trees were finally sprayed on May 20 and 23, but at this time the leaves were for the most part unfolded, although in varying degree.

Only DDT was used as an insecticide. The various formulations employed included an emulsion, a solution and a suspension (wettable powder). The concentrations of DDT used per gallon were 0.5, 1.0 and 2.0 pounds for the three formulations. Each formulation at each concentration was applied to the trees at dosages of 0.25, 0.50 and 1.0 gallon per tree, respectively. A total of 27 elms was sprayed. Well separated trees were chosen for treatment to avoid contamination by spray drift as each treatment was made.

Subsequent crotch feeding tests made about one month after spraying indicated that none of these treatments afforded what is considered to be control level; that is, no crotch punctures which penetrate to the vessels, in twigs from the upper crown. The same results obtained in the center crown, while the lower crown was satisfactorily

protected in a few cases. These variations in results could not be correlated either with type of formulation, or with the concentrations and dosages used.

On September 4, four trees on Canner Street, New Haven, were sprayed with the mist blower. Only an emulsion was used, and the DDT concentration was 1.0 pound per gallon. Two trees received a dosage of 2.5 gallons, and two trees received 5.0 gallons each. The trees were still in foliage and their height was estimated at about 70 feet. Tests made one day after spraying showed no crotch feeding scars on twigs from the latter two trees, even from the top crown, while the first two showed six and three scars, respectively. Checks showed from 5 to 35 scars. These results indicate that with the equipment used these dosages may give satisfactory protection.

It was obvious that the failure of the treatments in the Edgewood Avenue plot was due chiefly to the low dosages employed. This resulted in lack of adequate deposit on the twigs in the most vulnerable portion of the tree, the upper crown.

C. EXPERIMENTS IN 1948

The work carried out on this problem in 1948 was influenced by the findings of the previous year. It was decided to select a block of trees in an area of intense infection, and to spray them each year for several years. It is hoped that this program will determine whether or not such treatment will protect large trees in a given unit area. Trees of a large size were considered necessary, since it is this type of tree which is in direst need of protection.

It was also planned to obtain data on the relative effects of different concentrations of DDT, on the dosages necessary to cover trees of different sizes, and on the number of spray applications needed per season.

Only one spray formulation, an emulsion, to be used in the mist blower, was decided upon. Suspensions (wettable powders), in general, lack the sticking qualities of emulsions. Solutions, in the high dosages required in this particular problem, may be injurious to plant tissues.

An area was chosen in the Westville section of New Haven, as it offered an ideal combination of all of the factors desired. A high incidence of disease is present, the trees are about as large as are found generally in the city, and they are so located that they can be considered as a unit. They consist entirely of street trees, that is, trees situated between the sidewalk and the curb. The spacing of such trees is approximately forty feet.

All of the trees to be included in this experiment were selected while still in the dormant condition, and were listed by street and house number. The diameter breast high of each tree was taken with a diameter tape, and the height was measured with an Abney level and 100 feet from the base of the tree. A longer horizontal distance

might have given greater accuracy for a tree of this crown type. However, because of the interference of houses and other trees, it was not possible to use a longer distance and still see the top of the tree being measured. The trees to be sprayed numbered 110, and the check trees 103.

Certain trees had to be discarded from both spray and check plots, as will be explained later. The spray trees remaining ranged in diameter from 7 to 40 inches, with an average of 26.19 inches, standard deviation ± 7.81. The height of the spray trees ranged from 28 to 78 feet, with an average of 59.65 feet, standard deviation ± 10.33.

The diameter range of the check trees was from 6 to 37 inches, averaging 22.27 inches, standard deviation ± 7.31. Their heights ranged from 31 to 70 feet, with an average of 53.03 feet, standard deviation ± 8.20.

A 12 per cent DDT emulsion was used as a dormant spray on most of the experimental trees. This formulation contains the following per gallon of final mixture: DDT (technical grade), 1.0 pound; Xylene (industrial grade), 2.25 pints; Triton X-100, 1.5 ounces; water, 5.6 pints. Ninety-three of the trees were sprayed with this concentration and 17 with a 6 per cent emulsion.

Spraying was started at an early hour because the air is usually calm at that time. It was possible to work an average of about three hours each morning before it became necessary to stop because of wind movement. Application of the dormant spray commenced at daybreak (about 6:00 A.M.) on April 26, and the operation was finished on April 29. The elm buds were just beginning to break open on the final day. A modified Bean "vapo-duster" was used with "Whirljet" spray nozzle No. 1/8A2, orifice 5/64 inch, with an output of about one gallon per minute.

In view of the poor results obtained in the previous year, predetermined dosages were not used. Instead, each tree was sprayed until it was empirically determined that the tree was thoroughly covered. Then the dosage which that tree had received was measured and recorded. The average dosage, based on all trees receiving a measurable quantity, was approximately 4.2 gallons (4.2 pounds of DDT) per tree.

A second spray application was made on July 20, using only a 6 per cent emulsion. Since one objective was to determine the number of sprays necessary, only half of the trees receiving the dormant sprays were treated at this time. With certain restrictions, the groups of trees sprayed were randomized from the original block. Arbitrary decisions were unavoidable in certain cases. It was necessary to separate the spray trees from those which were not to be sprayed in such a manner that the trees in the latter group would not be contaminated. Fifty-nine trees were so selected, approximately one-half of the total number of experimental trees. These were divided in such a way that 50 trees had received the dormant spray consisting of a 12 per cent emulsion. The remaining nine trees had previously received the 6 per cent emulsion.

The spraying was done with the same equipment and was completed in one morning between 5:30 A.M. and 10:30 A.M. The dosage applied to each tree was measured and recorded as before. For all trees receiving a measurable amount, the average dosage per tree was approximately 2.6 gallons (1.3 pounds of DDT).

At the time when the elm leaves unfolded in early May, it was observed that a number of both spray and check trees contained Dutch elm disease "flags", that is, groups of typically discolored and wilted leaves. Since no bark beetles, or signs of their feeding had yet been noted, it was suspected that these trees had become infected during 1947. Such trees were recorded and were examined for year of infection on August 13.

The year of infection was traced by cutting out affected branches of various sizes. These were then cut and faced beyond the sections which had been dead long enough to have become colored entirely brown. At the satisfactory level, the wood was smoothed by a sharp cut, and was examined with a hand lens. The extent of discoloration could then be seen easily. Of 11 spray trees thus examined, it was found that eight had definitely become infected the previous year. It is believed that two of the remaining three were likewise infected in 1947. However, discoloration in the wood of that year could not be positively established, but was found only in the 1948 wood. One tree, in addition to discoloration in 1947, also showed staining in the 1943 wood. The rings of the intervening years were unstained.

It should be noted that seven of the eight trees infected in 1947 showed staining only in the late, or summer, wood of that year. Only one tree had staining in the 1947 spring wood. This is particularly interesting in view of the fact that elm trees are considered to be much more susceptible to infection during the period of early, rapid growth.

Eleven of the check trees were examined in a similar manner and, of these, three had staining in 1947 wood. Two of the latter also were infected in 1946. The spray trees and the check trees which were found to have been infected previous to 1948 were discarded from the experiment, except for purposes of dosage calculations.

D. RESULTS

Both spray and check trees were examined at intervals during the summer for visible indications of infection. They were last examined during the first week of September. At this time, only three or 2.94 per cent of the corrected total number of sprayed trees were definitely infected. These were the same trees mentioned in the previous section. Two of the trees had shown extensive wilting by late June, with practically the entire crown involved, and were suspected of infection previous to 1948. Twenty-one of the check trees, or 21.0 per cent of the revised total number were infected. Because of the small number of sprayed trees which became infected, it was impossible to distinguish possible differences between number and timing of applications, and between insecticide concentrations used.

A period of intensely hot, dry weather during the latter part of August and early September affected the elm foliage so severely that subsequent inspections were impossible. The second brood of bark beetles was not at peak emergence in New Haven until the week of August 29-September 4. This brood was three to four weeks later than that of the previous year in appearing. The delay was in consequence of the deferred peak emergence of the spring or first brood of beetles, which occurred during the week of June 22-June 28. As a result, the effect of the second brood upon the experimental trees could not be ascertained. This will be done as soon as the trees are in leaf in May, 1949.

In this connection, an attempt was made to determine whether or not peak emergence is dependent on air temperature. Wallace and Beard (75) have shown that elm bark is not a good insulating material, except against sudden and momentary temperature fluctuations. Weather data for months of March, April, May and June were used for the first brood. There appeared to be no correlation. However, when the accumulative hours of actual sunshine were used, there was a definite trend. The same trend appeared for the second brood when the months of July and August were included with those months previously listed. A direct relationship between accumulated hours of sunshine and rate of development of this bark beetle appears to exist. It may be that insolation, rather than air temperature, is one of the more important factors in determining peak emergence date.

Unfortunately, records of peak emergence for only three years were available. It will be necessary to obtain such records for several additional years and from various localities, before a true correlation can be shown or disproved.

E. RELATION OF DOSAGE TO TREE HEIGHT AND DIAMETER

One of the questions which arises frequently in connection with the spraying of elms is that of correct dosages for trees of differing size. It has been pointed out in the introduction that in this problem, adequate coverage of the small twigs which are susceptible to bark beetle feeding is most necessary. The question is particularly important if spraying is to be done with a mist blower. This type of equipment is comparatively new, and its qualities are in the process of being explored. Small amounts of spray material, compared with those used in hydraulic spraying, are involved, and they are more difficult to gauge correctly.

How, then, may the amount necessary to cover a tree of a given size be determined? It is, of course, crown volume which is to be sprayed; hence, a means of estimating this quantity is needed. To be of practical significance, the method must be simple and capable of rapid calculation.

Studies of the various factors affecting and influencing tree growth, particularly from the forestry standpoint, have shown that the size of the live crown is reflected in diameter growth (12, 32, 49). This

relationship does not hold for height growth. It must be borne in mind that spacing affects crown size and form and that an isolated, open-grown tree will differ considerably from a tree growing in association with other trees.

Wolfenbarger (82) has shown that the rates of increase in the number of twig crotches are somewhat similar to rates of increase of trunk diameter, regardless of tree location. His work was based on small trees of one to four inches. However, the average number of crotches per tree differed significantly with location. The number of crotches in elm branches of different diameters exhibited the same relationships. Generalized formulae for computing number of twig crotches based on diameter, therefore, could not be applied.

As stated on p. 52, all of the trees in the Westville plot are subject to the same environmental conditions. An exploratory treatment of the data was made to determine whether or not a usable criterion for estimating dosage could be obtained from it. The 100 trees receiving a measurable quantity of spray material during the dormant application in the Westville experiment were first grouped by five-inch diameter classes. Then the mean dosage for each class was plotted against its diameter interval. The connected points, weighted by number of items, showed a linear trend, and it appeared that further treatment of the data would be in order. A similar chart was made, based on height classes at five foot intervals, but the plotted points were widely dispersed.

A study was made of the regression of dosage on trunk diameter only and of the added effect of tree height.[1] The added effect of height clearly is not significant.[2] Dosage, therefore, can be based on trunk diameter without interference due to height.

The line showing the relation of dosage in gallons to diameter in inches was calculated. At the average trunk diameter of the sprayed trees, 27.70 inches, the dosage as read from the line is 4.169 gallons. The line defined by the equation[3] expressing this relationship is plotted in Figure 9 with the mean observed values. That portion of the curve below 12 inches is not reliable and has been substituted for by the dotted line inserted.

It would appear that where environmental conditions are quite uniform, as in the Westville plots, diameter is the best criterion for estimating individual tree dosage, and that a formula describing this relationship can be applied. This formula cannot be used for open-grown trees, or for trees in close position.

The dosages indicated are not intended to be absolute. They are merely suggested as a base line from which to work, but should be considered as minimum dosages. In the upper diameter range, particularly, a tree may absorb more spray material than is indicated

[1]The writers wish to thank Dr. C. I. Bliss for his advice and assistance on this problem.

[2]With $n_1 = 1$, $n_2 = 99$, $F = 3.94$, and an F ratio of 0.169.

[3] $Y = a + b\,(x - 27.70)$; where Y = dosage sought; a = 4.169 gallons; b = slope of line, 0.19; x = diameter of tree to be sprayed.

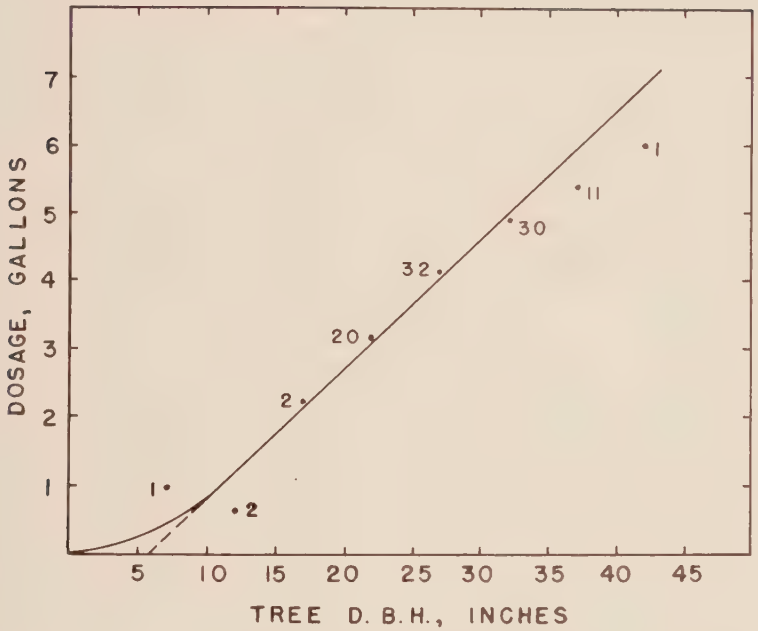


Figure 9. Relation of dosage to tree diameter, for dormant spray application. Plotted points represent number of trees observed in each 5-inch diameter group.

by the graph. The dosages are subject to future change when additional data is available. However, it is considered that they are reliable enough at present to be of assistance in a spraying operation. In addition, by use of this chart, the amounts of material needed can be roughly gauged, if the total number of trees and their average diameter is known.

If a summer spray is to be directed against the second brood of bark beetles, it is suggested that for each tree approximately the same dosage be used as was applied during the dormant period. At this time, the interference imposed by the screen of leaves must be taken into account. Much of the material which would otherwise be deposited on the twigs is intercepted by the leaves.

F. CONCLUSIONS

The results of the work done on this problem in 1948 show promise that feeding by *Scolytus* beetles and subsequent infection by Dutch elm disease from this cause, may be prevented in many cases by thorough application of the proper DDT spray. This is substantiated by the as yet unpublished findings of other research agencies.

Despite the progress made, many unanswered questions still remain. Some of the problems encountered have been outlined in the

introduction. In addition, there are others. Outstanding among these is the evaluation of other vectors, notably *Hylurgopinus rufipes* (48, 53). In Canada, where the activities of this insect are not masked by the presence of *Scolytus*, it has proved to be a most effective carrier of the disease. It must be determined how control of this vector, if necessary here, can be fitted into the spray program.

It must be borne in mind that absolute protection cannot be gained merely by a spray program. The latter should be supported by a sanitation procedure aimed at reducing the available breeding material and, hence, the beetle population, in a given area. Spraying will not prevent infection by means of root grafts between diseased and healthy trees.

The results of the first year of a spraying program in an area of high infection rate will be disappointing. The trees are sprayed when dormant and it may be impossible, without a detailed examination, to distinguish healthy trees from those already infected. A continued high tree death-rate during the first year of treatment may, therefore, be incorrectly blamed on failure of the spray application to protect, rather than upon this unavoidable complication. However, if the trees within a given area are sprayed regularly and carefully, and all possible breeding material is also treated, there should be a marked reduction in beetle population, and in the number of infections in succeeding years.

This type of spraying may be considered expensive. In a relative sense, it is not. Tree and stump removal in an urban community is a very costly operation. When money is spent in this way, there is nothing to show for it. The tree is gone and cannot be replaced for several decades. A tree can be protected for many years for what it would cost to remove it, were it to become diseased.

IV. The Value of Combining Chemotherapy and Vector Control by DDT

A. LIMITATIONS OF TECHNIQUES APPLIED SEPARATELY

Two experimental techniques of attacking Dutch elm disease have been considered in this paper. Let us explore what factors will limit the level of control from each of them, and consider in light of this what may be done to obtain maximum levels of disease control.

1. Vector Control by DDT Sprays

Up to now, DDT applications have been designed to operate against the principal insect vector in this area, *Scolytus multistriatus*. For this use it appears to offer great promise. However, experiments have adequately demonstrated that much better coverage of the tree by DDT and much more DDT is necessary to control crotch feedings by *Scolytus* than to control leaf-eating insects. Thus, a tree may have an amount of DDT adequate to eliminate feeding of the canker worm and the elm leaf beetle for practical purposes, yet be poorly protected against crotch feeding by *Scolytus*. If control of *Scolytus* to a practical degree is to be attained under field conditions, very high standards must be adhered to for proper coverage.

A second insect vector is the native elm bark beetle, *Hylurgopinus rufipes*. So far as is presently known, this is the primary vector in spreading the disease in the St. Lawrence River Valley. From the way in which the disease has spread there, we have revised our estimate of the importance of this insect as a carrier. It is known to bore into the bark of branches and trunks of trees (48). By this activity it carries spores of *C. ulmi* to the tree and causes infections there where the bark is thin (53). Presumably infections can occur only where the bark is thin because this bark beetle does not habitually bore deeply. If so, probably it introduces spores into branch wood. We do not know to what degree this insect walks on the bark surface before it bores into the tree. Probably the insect is more susceptible to DDT than *Scolytus* is (52, 67). However, we do not know if the amounts of DDT deposited on the upper surface of branches, especially in crevices in the bark, are adequate to prevent entry there. If timing of application, deposit of insecticide or habits of the insect are not within the critical limits, then DDT applications will not provide control of infections caused by this insect.

Another factor will limit the degree of control attainable by controlling the insect vectors. What is being done is to attempt to control disease by controlling the insect which spreads it. It does not follow that, because an insect is controlled to 50 per cent of its former level, the disease which it carries will also be controlled to that extent.

The numerical relation in a particular case will depend upon the absolute level of insects, the number of diseased trees or centers of infection in an area, the abundance of elms and their distribution. The disproportionality between insect control and disease control becomes accentuated in the case of a systemic disease, such as Dutch elm disease. In such a case, a tree, which becomes infected through the feeding of one beetle carrying *C. ulmi*, becomes diseased and it matters little whether only one or thousands of fungus-carrying beetles feed in the tree.

Finally, Dutch elm disease may be carried from tree to tree by agencies other than insects. Against these DDT sprays will be of no value. Verral and Graham (74) have shown that root grafts between adjacent elms, one of which is diseased, will permit passage of the fungus to the healthy tree. The roots underlying a stump remain alive for several years, as evidenced by sucker growth. Even though a diseased tree be removed, the living roots may form grafts with healthy ones and the disease thus be transmitted. It would also appear possible that spores of *C. ulmi* are air blown in nature. Smucker (62) has demonstrated this and shown them to be viable after being carried as far as 40 feet. He further states: "Other experiments indicate that should viable spores lodge in a favorable place, such as in a fresh wound in an elm tree, infection may occur". The conclusion that this is a significant source of new infections cannot be drawn (31), yet, in view of the experimental evidence, it remains a possibility.

Rain water is another possible means of disseminating spores of *C. ulmi*. Tyler, Parker and Pope (73) have indicated that *C. ulmi* may bear spores superficially on trees, that rain water may carry the spores for some distances, and that, when these spores lodge in wounds up to two weeks old in roots, stems or branches, infection of the tree may result. While it is not likely that this is important, except in cases of adjacent trees, it assumes significance when protection of the valued tree is considered.

By way of summary, then, one concludes that new infections of Dutch elm disease may be anticipated despite the best use of DDT sprays that can be made. Protection cannot be assumed to be absolute, although protection will be far more sure than with no treatment.

2. Chemotherapy of Healthy Trees

The many limitations of this technique have been discussed already and need only be summarized here. With present materials and techniques of applying them, it would appear that protection is somewhat less than results from vector control with DDT sprays. The amount of protection appears to be related to the inoculum potential. The more disease in an area and the more bark beetles to bring inoculum to a tree, the less will protection be.

The chemotherapeutic method is designed to operate against the fungus causing the disease, however it may be brought to the

tree. Moreover, chemotherapy operates internally within the plant. The chemotherapeutic agent will become less effective with time inside of the plant. It may become less effective through chemical reaction, through dilution as it becomes distributed into the leaves, through being left behind as the tree grows in girth, or through being dropped with the leaves in autumn.

B. VALUE OF A COMBINED PROGRAM

Enough is known of the mechanisms by which vector control and chemotherapy work to indicate that these practices operate to control Dutch elm disease independently of one another. One operates against the insect vector which spreads the disease; the other operates against the fungus which causes the disease. Ordinarily plant pathologists must be content with a single primary means of combating a plant disease, and the situation presented here is somewhat novel in this regard. It is reasonable to inquire what value accrues from using both techniques together in a control program. In general, when two procedures operating independently of one another produce the same result, the combination of the two into one program produces far greater an effect than either procedure by itself. In the present case, if we know the probability that a tree will become diseased despite treatment by either method alone, then the probability that a treatment will become diseased under a combined program will be the product of these two probabilities. We do not yet know the probabilities in this immediate case, but a numerical example will illustrate this thought. If two trees of 100 sprayed with DDT become infected in any one year, the probability of infection is 0.02. If five trees of 100 treated with OQB become infected under these conditions, the probability of infection is 0.05. Under a combined program, the probability of infection should be 0.02 x 0.05 or 0.001. This means that only one tree in 1,000 will become infected under a combined program, if the probabilities assumed are correct. It must be emphasized again that the values given here are assumed and are not experimentally deduced. They have been used merely for illustrative purposes.

On the basis of such reasoning, an experiment has been set up in which these two methods of control are being compared, separately and together, on small, newly planted street elms in collaboration with the Park Department of the City of New Haven. The 260 trees average two inches in diameter at breast height. These were divided into four lots of 65 trees each and will receive four different treatments: (1) DDT alone, (2) OQB alone, (3) both treatments, and (4) neither treatment. In 1948, the first year of the experiment, DDT was applied to all trees in the experiment to protect them from elm bark beetle while they were becoming established. Actual applications in accordance with the above schedule will be first made in 1949. OQB was applied in 1948 as specified above. In the first year, 1948, three trees became infected with Dutch elm disease. None of these had received applications of OQB. On the basis of such experiments, and in terms of small trees, the probabilities of infection by the two techniques separately and together can be determined.

V. Summary

1. Dutch elm disease has become firmly entrenched in Connecticut and will not be reduced significantly by sanitation on a large scale or by eradicative efforts.

2. Chemotherapy promises to become a new technique for controlling vascular wilt diseases, of which Dutch elm disease is an example. A possible means of chemotherapeutic action consists of inactivating the toxins produced by vascular wilt fungi. Toxins can be better inactivated if they are better known. Studies were undertaken to isolate the toxin of *Ceratostomella ulmi* from metabolism solutions.

3. Instead of one toxin, a series of phytotoxic fractions has been isolated. One of these is a polysaccharide or related complex carbohydrate. Another suspect is gluconic acid. Others as yet unidentified have also been separated. Some of these cause wilting of the leaf and some cause necrosis when they are absorbed into tomato cuttings. They produce similar symptoms on elm cuttings also.

4. The bearing of toxins from cultures *in vitro* upon pathogenesis is discussed, and the need for studies carried out *in vivo* to distinguish real from apparent toxins and to aid in chemical identification of toxins is urgent.

5. Resistance to toxins is apparently not the basis of resistance to Dutch elm disease in *Ulmus pumila* and the Buisman elm.

6. The principal toxins isolated in these studies severely affect the water economy of cuttings placed in them. *Ulmus pumila* is shown to have greater resistance to physiological wilt than *Ulmus americana*.

7. As applied to chemotherapy and the selection of chemotherapeutants, the finding of several toxins poses a serious problem. Several toxins, differing chemically, will require several chemotherapeutants to inactivate them. It is probable that the chances of successful absorption, distribution in the plant, and inactivation of toxins there are very much less if several toxins must be inactivated than if only one must be acted upon.

8. Chemotherapeutants may act in the plant in a number of ways. It seems desirable to design tests so as to select compounds having chemotherapeutic activity, regardless of how such activity is brought about. Such tests have been designed and consist of treating plants with chemotherapeutant, inoculating the plant with a pathogen, and measuring the degree of disease control obtained.

9. 8-Quinolinol benzoate was selected as a compound with which to explore the principles of field chemotherapy on Dutch elm disease. The compound was principally applied to the soil in solution about feeding roots, and the plant absorbed the chemotherapeutant from the soil solution.

10. Dosage of chemotherapeutant should be increased in direct proportion to diameter of the tree in applying chemotherapeutant to a large tree on the basis of information obtained from small ones. Great care must be taken in establishing the chemotherapeutic dosage for a small tree, since an underestimate will be an error which is multiplied many times in estimating the proper dosage for a large tree.

11. With 8-quinolinol benzoate, the ease with which cultures of *C. ulmi* may be recovered from treated and inoculated trees is related to the amount of disease expressed in terms of crown involvement. This is a result of chemotherapeutic treatment.

12. Used as a *curative* measure on trees already showing disease symptoms, 8-quinolinol benzoate suppresses symptoms of disease, but in general does not prevent trees from dying in practice. Differences in severity of disease as measured by the percentage of the crown of the tree showing symptoms can be established and maintained in treated plots when compared with untreated plots. This effect is most clearly demonstrated on small trees.

13. The larger a tree is, the more likely is it to die of Dutch elm disease despite treatment. This effect results from the fact that Dutch elm disease is far more severe on a large tree than on a small one. The relation is probably related to rate of radial growth of the tree. A small tree probably grows radially faster than the fungus can grow through cell walls. If healthy tree cells are laid down outside the invaded cells at a sufficiently rapid rate throughout the tree, the infection is walled off. In a larger tree this apparently does not occur.

14. The value of a treatment lasts no more than one year.

15. Applied to the soil as a solution, 8-quinolinol benzoate is most effective when applied with an injection nozzle under pressure from 18 to 24 inches beneath the surface of the soil in the feeding root zone. Some value of treatment accrues from trunk injection of solutions, but the effect is erratic, probably owing to poor distribution of the chemotherapeutant.

16. As a preventive measure, chemotherapy can be demonstrated to protect the tree against infection. Protection is not absolute, however. It appears that protection is greater when inoculum is low than otherwise.

17. Applications of chemotherapeutant timed to coincide with emergence of bark beetles appear to be more effective than other schedules of application.

18. Feeding by *Scolytus* beetles and infections by *Ceratostomella ulmi* consequent to this may be prevented in many cases by thorough application of the properly formulated DDT spray. The value of such a program has been explored, using a mist blower for applying the sprays.

19. In 1947 a series of dosages of DDT was applied to elm trees with a mist blower, and value of treatment was estimated in terms of the number of successful crotch feedings by *Scolytus*. These tests indicated that high dosages of DDT and very thorough coverage, especially of the upper crown, are mandatory for successfully preventing crotch feedings.

20. In 1948 a plot of 110 elms was treated with a mist blower. The first application was made in late April. Ninety-three of the trees were sprayed at this time with a 12 per cent emulsion and 17 were sprayed with a 6 per cent emulsion. Half of these trees received a second application with a 6 per cent emulsion of DDT on July 20. A plot of 103 unsprayed trees served as a check.

21. Only three trees in the sprayed plot became infected in 1948 whereas 21 trees in the unsprayed plot became infected. In both plots the trees infected before 1948 were eliminated by removing twigs showing symptoms of disease and noting in what year vascular discoloration began.

22. Because very hot dry weather in late August seriously affected elm foliage, and because this weather preceded the peak of emergence of the second brood of bark beetles, the effect of this brood could not be determined in 1948.

23. There appears to be a relation between the accumulative hours of sunshine and the rate of development of the elm bark beetle. The cumulative hours of sunshine may be one of the chief factors determining the peak emergence date.

24. The relation between diameter of a tree and the volume of spray material required for adequate coverage is linear. A curve showing this relation is presented in terms of the plot sprayed in 1948.

25. Other sources of infection may or may not be affected by applying DDT sprays. *Hylurgopinus rufipes*, a second insect vector, is the only other known source of infection which is likely to be seriously affected by DDT. How successfully DDT will control it has not yet been evaluated. Other sources of infection will not be affected by DDT. These include root grafts between healthy and diseased trees, and possibly lodging of spores in wounds, carried there by wind and rain.

26. Protection by chemotherapy or by vector control is not absolute. Trees will become infected in spite of treatment by either method. These two methods of treatment operate independently of one another, one acting against the fungus causing the disease and the other acting to prevent the insect vector from successfully feeding in the tree and thus, carrying the fungus there. It seems logical, therefore, to combine the two control measures into one operation, where possible, since levels of protection ought to be increased thereby.

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